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FUNGI CAUSING STAIN IN LOGS AND LUMBER IN THE SOUTHERN STATES, INCLUDING FIVE NEW SPECIES [1]

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INTRODUCTION

The sapwood of many important pine and hardwood species is subject to discoloration caused by fungi. The most common stain is one of a bluish-gray hue which often occurs in spots, streaks, or patches, or which may, under suitable conditions of moisture, involve the entire sapwood area of logs and lumber. Such discolorations have been the source of serious losses to manufacturers of wood products, particularly in the Gulf States region. The present investigation was undertaken in July and August 1931 and continued through May and June 1932 to determine more definitely what fungi were chiefly responsible for such staining of wood in the southern United States. Cultural and identification studies were conducted later in the laboratory.

REVIEW OF LITERATURE

While a number of fungi have been described as causing stains in the United States, no systematic studies of their relative prevalence have been made in the field. In 1903 Von Schrenk (*17*)[3] investigated the staining of pines in the Black Hills section and attributed the cause to a fungus which he called *Ceratostomella pilifera* (Fr.) Winter. In 1906 Hedgcock (*7*) published descriptions of 20 fungi which he had found capable of staining wood. In 1929 Rumbold (*14*) also showed, as a result of many isolations from stained wood, that there are a number of species responsible for staining. In the same year Nelson and Beal (*12*) gave the results of inoculation experiments in which they used three types of staining fungi which they found associated with bark beetles. The following year 2 of these 3 fungi were described by Rumbold (*15*), 1 never having been reported before in North America and the other being a new species. A number of the fungi mentioned by Hedgcock and Rumbold were Fungi Imperfecti, but *Ceratostomella* species have long been considered by workers in this country to be responsible for most of the staining of logs and lumber. However, Lagerberg, Lundberg, and Melin (*9*) state that in Sweden species of the Fungi Imperfecti were more important as stainers than were the species of *Ceratostomella*.

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[2] The writer is indebted to Caroline T. Rumbold, of the Division of Forest Pathology, for identification of many of the fungi isolated during the first part of the investigation, and to other members of the Division staff for advice as to methods of conducting the investigations in the field. The Latin descriptions were prepared by Edith K. Cash, Division of Mycology and Disease Survey. The writer is also indebted to Neil E. Stevens, of the Division of Cereal Crops and Diseases, for identification of the species of *Diplodia*.

[3] Reference is made by number (italic) to Literature Cited, p. 806.

METHODS

Freshly sawed, unstained sapwood boards were taken as they came from the sawmill and placed in newly erected stacks of lumber in the seasoning yard. Care was taken that these samples were placed where they would not dry too rapidly and thus prevent the appearance of stain. From time to time small sections were sawed from these boards and taken in paper bags to the laboratory, where an attempt was made to isolate any fungi that had grown into the wood. The transplants to agar were normally made on the same day, and almost invariably within 24 hours.

After finding that the presence of molds frequently prevented the isolation of staining fungi from lumber that had remained in the yards for 1 to 3 months or more, it was decided that, for the present study, only those stained samples which had been in the yard for 3 weeks or less would be used. The following discussion of the lumber-staining fungi and the tables giving the frequency of their occurrence are based on isolations from such freshly stained lumber samples.

At Natalbany, La., and Caryville, Fla., a chemical treatment was being used to prevent staining. There undipped boards were placed beneath the stacks of lumber to avoid direct contact with the dipped lumber. Where this was done, it was found necessary to place several test boards together, one on top of the other, in order to prevent too rapid drying.

The wood samples were from the following common tree species used for lumber in the Gulf States: Longleaf pine, (*Pinus palustris* Mill.), shortleaf pine (*P. echinata* Mill.), loblolly pine (*P. taeda* L.), red gum (*Liquidambar styraciflua* L.), yellow poplar (*Liriodendron tulipifera* L.), tupelo (*Nyssa aquatica* L.), beech (*Fagus grandifolia* Ehrh.), magnolia (*Magnolia* sp.), oak (*Quercus* sp.). At the pine mills longleaf pine was the most common species used, and at the hardwood mills red gum, oaks, and yellow poplar, probably in the order named, were the more important.

In the laboratory the wood samples from which transplants were to be made were split open and immediately placed in a culture chamber. Then small particles of wood were removed from beneath the freshly exposed surface and planted in Petri dishes containing 2-percent malt agar. These particles were removed with a "chisel forceps", which was dipped in alcohol and flamed each time after it touched the freshly exposed surface of the wood. It is possible that contamination resulted occasionally by air-carried spores, but results indicated that this was seldom the case. In making isolations from "yard-stained" lumber,[4] which had molds fruiting on the surface, all of the outer surface was removed in a separate room, or alcohol was sometimes swabbed over the surface and flamed off. However, the results seemed about the same whether these additional precautions were taken or not.

If a sample was unstained or very slightly stained, isolations were made from near the surface, but if it was heavily stained, some were made from a greater depth and others from near the surface. In either case it was difficult to make isolation from a depth less than

[4] By "yard-stained" is meant stain which originated after the lumber was sawed, as distinguished from log stain.

one-eighth to one-sixteenth inch without danger of obtaining surface contamination.

Most of the stained boards contained an abundant growth of perithecia of species of *Ceratostomella* on the surface. Isolations were made from these to determine whether the species fruiting on the surface were the same as those obtained from the interior of the wood. This was done by touching the droplets of ascospores, which had collected at the tips of the perithecial beaks, with a flamed platinum needle and streaking them over poured plates of malt agar. *Graphium* was frequently isolated in a similar way.

In order to determine whether the organisms causing stain in the logs were the same as those causing stain of lumber in the mill yards, the lumber was watched as it came from the sawmill and samples were taken from those already stained. Logs were also examined before they reached the sawmill and samples were taken from any that were stained.

All fungi that grew from the wood were transferred to test tubes and studied later in the laboratory. These studies were made on 2½-percent Trommer's malt extract agar and the growth rates were obtained at a room temperature of about 75° F.

OCCURRENCE OF THE FUNGI

FUNGI ISOLATED FROM PINE LUMBER

Freshly sawed pine lumber, which was placed in the mill yard to dry, showed a few small spots of stain after 3 or 4 days under humid conditions of weather. If the weather was very dry the boards remained free of stain. If the lumber was solid-piled for 2 or 3 days, either along the conveyor chain at the mill or on the runways in the yard, it became at least slightly stained in the driest weather, and if bulked for longer than 4 or 5 days the entire sapwood became badly stained.

Provided the weather was favorable for stain development, as it is much of the time in the Gulf States, the fungi were isolated from near the surface within 3 or 4 days after the lumber had been taken from the sawmill. After a period of 5 to 7 days they could usually be obtained from the center of a 1¼-inch board.

The organisms responsible for the staining were found in such abundance that several species were usually present in each sample. Even when only 1 staining fungus was obtained from the interior, isolations made from the perithecia fruiting on the surface showed that 1 or 2 others were usually present. This made it difficult to associate staining with any particular organism.

The fact that several fungi were isolated more frequently than others does not necessarily prove that they are of first importance as stainers. In the first place, no pure-culture inoculations were made to determine the relative staining ability of the several species concerned. Secondly, their rate of growth in culture differs considerably, and a rapid-growing species might often appear in culture to the exclusion of the others. *Ceratostomella ips* Rumbold, for instance, grows more rapidly than *C. pilifera*, and although the two can usually be detected when growing together, it is possible that in some instances *C. pilifera* was obscured. The *Ceratostomella* hereinafter described (p. 797) as *C. multiannulata* Hedgc. and Davidson, n. sp., certainly would often be suppressed by *C. ips*, *C. pilifera*, or *Diplodia natalensis* P. Evans.

The last-named species, which grows very rapidly, often suppressed all of the other staining fungi in the Petri-dish cultures.

In spite of these confusing factors, those fungi most often isolated from the interior of the stained samples seem to be responsible for most of the staining of lumber in the Southern States. This conclusion is more plausible when one considers the fact that the two fungi most frequently obtained, *Ceratostomella ips* and *C. pilifera*, are known to be severe stainers.

Table 1 includes a list of the fungi isolated from stained pine, and shows the number of times each fungus was isolated from pine lumber and logs in each locality where a study was made. In all of these localities except Caryville, Fla., sufficient samples were collected to give a good basis for determining which fungi were most frequently the cause of stain at that time. Only 6 days were spent at Caryville, which may explain in part the failure to obtain *Ceratostomella pilifera* there.

TABLE 1.—*Fungi isolated from pine and hardwood lumber and logs and their relative occurrence in several localities*

Fungus isolated	Number of isolations from lumber and logs in locality indicated											
	Bogalusa, La.		Urania, La., and vicinity		Laurel, Miss.		Natalbany, La.		Caryville, Fla.		Total isolations	
	Lumber	Logs	Lumber	Logs	Lumber	Logs	Lumber	Logs	Lumber	Logs	Lumber	Logs
FUNGI FROM PINE												
Ceratostomella ips	5	13	4	2	33	6	15	1	11	6	68	28
C. pilifera	6	3	2	3	20	3	14	2	0	0	42	11
C. multiannulata	0	0	0	0	5	0	0	0	0	0	5	0
C. pluriannulata	0	0	3	1	0	2	0	0	0	0	3	3
C. obscura	0	1	0	0	0	0	0	0	0	1	0	2
C. pini	0	0	0	0	0	1	0	0	0	0	0	1
C. sp.	4	2	5	4	0	0	0	0	0	0	9	6
Endoconidiophora moniliformis	0	0	2	0	3	0	1	0	0	0	6	0
Diplodia natalensis	2	3	0	2	6	2	6	2	2	1	16	10
Alternaria sp.	0	0	3	0	10	0	0	0	0	0	13	0
Helminthosporium geniculatum	1	0	7	0	0	0	0	0	0	0	8	0
Graphium rigidum	0	1	0	0	0	0	0	1	0	1	0	3
Diplodia megalospora	0	1	0	0	0	0	0	0	0	0	0	1
Cadophora brunnescens	0	0	0	0	0	0	0	0	0	2	0	2
C. repens	0	0	0	0	0	0	0	0	0	1	0	1
Hormonema pullulans	0	0	1	0	0	0	0	0	0	0	1	0
FUNGI FROM HARDWOOD												
Endoconidiophora coerulescens	6	8	9	8	3	5	3	8	1	1	22	30
E. moniliformis	0	0	12	5	2	3	3	4	4	0	21	12
E. fimbriata	0	0	0	0	1	0	0	0	0	0	1	0
Ceratostomella pluriannulata	4	3	9	9	0	1	0	0	1	0	14	13
C. sp.	0	0	2	2	0	0	0	0	0	0	2	2
Graphium rigidum	4	1	9	6	0	0	0	1	0	0	13	8
Diplodia natalensis	6	2	2	0	1	0	0	1	0	0	9	3
Leptographium microsporum	2	0	0	0	0	1	0	0	0	0	2	1
Hormonema pullulans	0	1	1	2	0	0	0	0	0	0	1	3

It was surprising that *Ceratostomella ips* was isolated more frequently than any other species, since it had previously been considered to infect only logs infested by the *Ips* beetle. It stains the boards dark gray-black, and occasionally large short-necked perithecia

form on the stained areas. Its great abundance in lumber is probably partly due to the fact that so many logs stained by *C. ips* pass through the lumber mills. Such logs and the products of their manufacture, as lumber and sawdust, probably serve as sources of infection to the unstained lumber in the mill yard.

Ceratostomella pilifera was isolated 42 times, which indicated it to be at least second in importance. It has usually been considered the cause of most of the lumber stain, and probably it is more important than *C. ips* in some localities. The surface of wood stained by *C. pilifera* is light gray at first, due to a dense formation of white conidiophores and conidia. This light-gray surface soon disappears, leaving a dark gray-black streak of stain, which is often as dark as that caused by *C. ips*.

Diplodia natalensis was isolated 16 times from stained lumber. This species seems to be of some importance in causing stain, although the samples from which it was obtained usually contained *Ceratostomella* also. It frequently infects the boards soon after they are placed in the mill yard and grows rapidly through the wood.

The unidentified *Ceratostomella* species were probably *C. pilifera* and *C. ips*. Other species of fungi grew so rapidly in culture with them that the species could not be determined.

Endoconidiophora moniliformis (Hedgc.) n. comb., *Ceratostomella pluriannulata* Hedgc., *Helminthosporium geniculatum* Tracy and Earle, and *Alternaria* sp. are possibly of little importance in pine, since they were never definitely associated with any staining during this investigation.

The remaining species, *Ceratostomella multiannulata*, must be placed in a doubtful position as to its staining ability. In those localities where a study was made in 1932, except at Caryville, Fla., this species was found fruiting in abundance on the surface of most of the sample boards. The fact that it was isolated from the surface of 70 samples and from the interior of only 5 indicates that it seldom penetrates deeply into the wood. Its perithecia often formed and matured quickly on unstained surfaces of the boards, but wood in which it grew almost always became at least slightly stained. Whether it caused any dark staining of the wood was difficult to determine, since other species were usually present soon after its appearance. Even if it were found to be of little importance in causing stain, it must still be considered one of the common fungi inhabiting freshly sawed pine lumber in the South.

FUNGI ISOLATED FROM PINE LOGS

Some of the logs that are brought to the mills in the South already contain much stained sapwood, and although this investigation was chiefly conducted to get information on the staining of lumber, a considerable number of isolations were also made from the stained logs. It was found that *Trichoderma* sp. and other molds which were so frequently obtained from lumber stained at the mill were seldom obtained from wood that had been stained in the logs.

As is shown by table 1, *Ceratostomella ips* was found to be the species responsible for most of the log stain; it always occurred in logs that had been infested with bark beetles (*Ips* sp.). *C. pilifera* was obtained from a number of samples, and its entrance, where definitely traced, was through broken or splintered areas of the log. The two

species of *Cadophora* were obtained only at Caryville, Fla., so they are probably of little importance as stain fungi.

Since *Diplodia natalensis* is so prevalent and grows on so many substrata, it is not surprising that it was occasionally obtained from logs as well as from lumber. *Ceratostomella pini*, which is known to obtain entrance along with bark beetles, was found only once. *C. obscura*, n. sp. (described on p. 797), was isolated only twice and may not cause much staining.

NON-BLUE-STAINING FUNGI ISOLATED FROM PINE LUMBER

A number of fungi other than wood-staining species increased the difficulty of obtaining the causal discoloring organisms in culture. At Bogalusa, La., in 1931, *Trichoderma lignorum* (Tode) Harz often appeared to obscure the growth of all other fungi. It penetrated the newly sawed boards as quickly as did the staining fungi and soon invaded all of the sapwood. At Urania, La., *Trichoderma* interfered less, but other fungi such as *Helminthosporium geniculatum* and *Diplodia* hindered the growth of *Ceratostomella* species to some extent. Because of these complications, the information obtained in 1931 was considered inadequate for any definite statement as to which organisms were chiefly responsible for the staining of pine lumber.

During 1932 the work was continued at Laurel, Miss.; Natalbany, La.; Caryville, Fla.; and Bogalusa, La. At all of these places except Bogalusa, *Trichoderma* and other nonstaining fungi interfered only occasionally. However, the rapidly growing *T. lignorum* again appeared from most of the samples obtained at Bogalusa.

Other non-blue-staining fungi frequently isolated were *Pestalozzia* sp., *Fusarium* sp., various species of *Penicillium*, and occasionally species of *Aspergillus*. Several species, *Helminthosporium geniculatum*, *Alternaria* sp., and *Endoconidiophora moniliformis*, are listed as doubtful staining fungi. *H. geniculatum* was obtained only at Bogalusa and Urania, La., and was always associated with species of *Ceratostomella*.

FUNGI ISOLATED FROM SPECIES OF HARDWOOD

Table 1 shows also the fungi that were isolated from stained hardwood logs and lumber. It shows that the fungi responsible for log stain occur in about the same relative frequency in stained lumber. Here the species of Ceratostomataceae are again of much greater importance than are the Fungi Imperfecti.

Endoconidiophora coerulescens Münch is of greater relative importance as a stainer of lumber than the number of isolations would indicate. It infects the ends of the logs soon after they are cut, and continues to grow rapidly over the surface of the lumber as soon as it is stacked in the yards to season. It forms a dense black surface growth of mycelium and perithecia, and the interior of the wood is stained gray. The staining is usually more uniform than the stain in pine and not so localized in streaks or spots. *E. coerulescens* grows on most of the species of hardwoods. It was isolated from sap gum, black gum, poplar, oak, beech, and magnolia, and probably occurs on many others. The odor produced by this fungus is distinctly noticeable wherever there is abundant infection of logs or lumber.

Ceratostomella pluriannulata and *Graphium rigidum* (Pers.) Sacc. also frequently inhabit hardwood logs and lumber. They were usually

found associated with *E. coerulescens*, but grow more slowly and seem to cause much less staining than the latter. The appearance in culture of *C. pluriannulata* is very similar to *G. rigidum*, and throughout this investigation the writer was unable to identify one from the other until fruits appeared. It is not known whether they are similar in their habit of growth in the wood and in their staining qualities.

Endoconidiophora moniliformis was found very frequently on hardwoods and occasionally on pine. The fact that it was not obtained at Bogalusa is difficult to explain, for it was a very conspicuous fungus in the other localities. Factors favoring it are its very rapid growth and the fact that mature perithecia are usually formed in 2 or 3 days. It causes a dark-brown stain in fresh sapwood, but this stain seems to fade out considerably when the wood dries thoroughly. It also is frequently found growing with one or several of the species previously mentioned.

Diplodia natalensis was isolated a number of times from hardwoods, but it was usually obtained from boards that had been first stained by *Endoconidiophora coerulescens*.

The other fungi listed are probably not of very great importance. *Hormonema pullulans* (De Bary) Lagerberg and Melin was considered by Hedgcock (*7*) to be the cause of stain, but in the present investigation it was usually obtained from very slightly stained boards.

In isolating fungi from recently stained hardwood logs or lumber, it was found that almost no nonstaining fungi appeared to interfere with the growth of those herein listed as possible staining fungi.

SYSTEMATIC POSITION OF THE BLUE-STAINING FUNGI

CERATOSTOMATACEAE

The foregoing account has been an attempt to show the relative importance of the wood-staining fungi that occur in the localities studied in the southern United States. In order that there may be little confusion concerning the identity of the species, it is essential to discuss them more fully. Complete descriptions of the well-known species are not included, but brief statements of their characteristics in culture are given.

The family Ceratostomataceae, which includes most of the important staining fungi occurring in the South, is a very unified group. The species have constant differences and may be easily identified when obtained in pure culture. In 1907 Münch (*11*) established the genus *Endoconidiophora*, which differs from *Ceratostomella* in that conidia are formed endogenously. This classification has not been followed by later authors. The type species, *E. coerulescens*, was the only one placed in this genus, despite the fact that *Ceratostomella fimbriata* (Ell. and Hals.) Elliott (*4*, *5*), *C. adiposum* (Butler) Sartoris (*16*), and *C. paradoxa* (De Seynes) Dade (*3*) have endoconidia. To this *Endoconidiophora* group will be added *C. moniliformis*, which has been found by the writer to have endoconidia. These endoconidial species constitute a group of closely related fungi. This may be emphasized by the fact that *C. moniliformis* was placed by the writer in the *Endoconidiophora* group before it was examined microscopically. The production of a distinct odor seems to be a character common to all of the species, and the three species that have been isolated from wood produce two rather distinct kinds of endoconidia, the one short, thick, and barrel-shaped, and the other narrow and cylindric.

Ceratostomella ips Rumbold

Rate of growth, 24 mm in 5 days.[5]

Ceratostomella ips has been adequately described by the author of the species (*15*). In culture it is the most rapid-growing species of

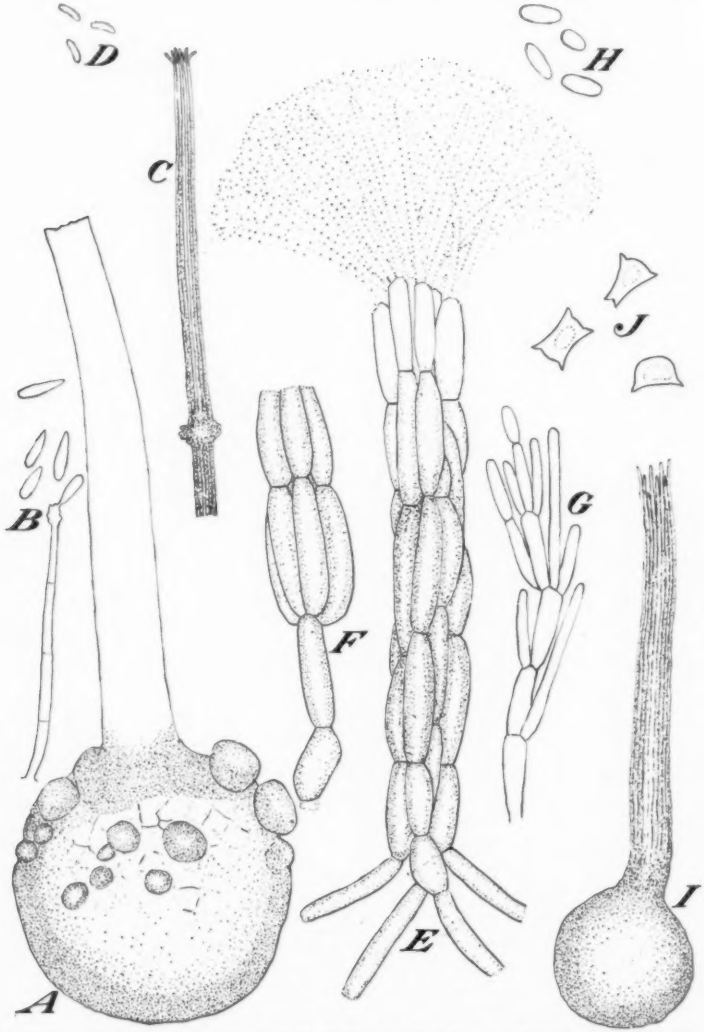

FIGURE 1.—*A–D, Ceratostomella multiannulata* Hedgc. and Davidson: *A*, Young perithecium (× 220); *B,c* onidiophore and conidia (× 1,000); *C*, tip of beak from mature perithecium (× 220); *D*, ascospores (× 1,000). *E–J, C. obscura: E*, Small synnema (× 1,000); *F*, base of small synnema (× 1,000); *G*, conidiophore originating directly on mycelium (× 1,000); *H*, conidia (× 1,000); *I*, perithecium (× 220); *J*, ascospores (× 1,000).

[5] Growth rates, where given, indicate the radial growth on 2½-percent malt agar at a room temperature of approximately 75° F.

Ceratostomella known to infect pine lumber in the southern mill yards. This characteristic, together with its dark-brown mycelium, makes it easy to identify soon after it appears in culture. The surface of the cultures usually becomes covered by a conspicuous growth of gray-brown matted mycelium and conidiophores, but in less vigorous cultures there is sometimes no apparent aerial mycelium.

Ceratostomella pilifera (Fr.) Winter [6]

Rate of growth, 14 mm in 5 days.

There is difference of opinion as to the validity of the name used for this fungus. Münch (*11*) considers it, as used by earlier mycologists, to refer to a group of fungi consisting of several distinct species. In North America, however, it has come to refer in general to the species described by Hedgcock (*7*) under that name.

The mycelium of *Ceratostomella pilifera* soon becomes smoke-colored and finally dark gray-black in the substratum. *C. pluriannulata* Hedgc. is the only other species studied by the writer which has mycelium of similar color and rate of growth, but it differs from *C. pilifera* in that segments or areas of the mycelium may remain entirely hyaline. The perithecia of *C. pluriannulata* also mature much earlier than those of *C. pilifera*, often forming on the hyaline mycelium as well as on the dark areas.

Ceratostomella multiannulata Hedgc. and Davidson, n. sp. (fig. 1, *A–D*).

Mycelio primum hyalino, brunnescente, demum obscure brunneo, supra e conidiis albo-pulverulento; peritheciis nigris, subdepressis, plerumque 160μ–260μ altis, 170μ–275μ latis, e cellulis obscuris globosis rugosulis; rostellis perlongis, 4–8 mm, e basi 48μ–65μ ad apicem 14μ–16μ in diam. attenuatis, 6–9 annulatis, circum ostiolum 12–16 filamentis brevibus rigidis filamenta 6μ–12μ longis praeditis; ascosporis hyalinis, paulo curvatis, 1.2μ–1.4μ×2.8μ–3.8μ, saepe conglobatis; conidiophoris hyalinis, 3μ–5μ latis, 70μ–180μ longis; conidiis 5–10 caespitosis, in apice inflato denticulato enatis, hyalinis, simplicibus, una extremitate acutis, 2μ–3.2μ×6μ–25μ.

Mycelium hyaline at first, becoming light brown in 4 or 5 days, and finally very dark brown, surface at first covered with a white powdery growth of conidiophores and conidia; perithecia begin to form when mycelium turns brown and mature in a day or two, are black, slightly flattened, 160μ to 260μ high by 170μ to 275μ broad, covered with dark globose cells which give a roughened appearance, necks very long, 4 to 8 mm, tapering from 48μ to 65μ thick at the base to 14μ to 16μ at the apex, multiannulate finally having 6 to 9 annulae, 12 to 16 short stiff filaments, 6μ to 12μ long, around ostiole; ascospores hyaline, slightly curved, 1.2μ to 1.4μ by 2.8μ to 3.8μ, often in bunches; conidiophores hyaline, 3μ to 5μ thick by 70μ to 180μ long; conidia borne at enlarged denticulate apex in groups of 5 to 10, hyaline, one-celled, pointed at one end, 2μ to 3.2μ by 6μ to 25μ.

Rate of growth, 10 mm in 5 days.

On pine lumber (nos. 59039 type, 59040, and 59041)[7] collected at Laurel, Miss., May 1932; Natalbany, La., (no. 59042) June 1932; and Caryville, Fla., June 1932.

This species was isolated and studied by Hedgcock in 1924, but a description of it has never been published. It differs from *Ceratostomella pluriannulata* in having much longer and more pluriannulate necks on the perithecia, in having the perithecia roughened with globose cells, and in having a more distinctly brown mycelium in culture.

[6] This fungus is possibly the same as the European species *Ceratostomella coerulea* Münch.

[7] These are numbers used by the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, and designate different strains of the fungi studied in culture. Transfers from all type cultures have been deposited in the mycological collections of the Bureau of Plant Industry.

None of the cultures obtained in 1931 was identified as this species, but samples of stained pine boards from which isolations were made in 1931 have perithecia of *C. multiannulata* on them.

Ceratostomella obscura, n. sp. (fig. 1, *E–J*)

Mycelio primum hyalino, ad substratum appresso, dein pallide griseo-viride, numquam obscurissimo; mycelio conidiofero fere invisibili, graphia mox formante; peritheciis tarde formatis, saepe absentibus, nigris, fere glabris, sphaericis, 90μ–150μ diam., rostellis 200μ–420μ longis, fere cylindricis, basi 30μ–45μ, apice 25μ–40μ diam., filamentis ostiolaribus setiformibus, 25μ–35μ longis, acutis; ascosporis membrana gelatinosa vestitis, reniformibus, 1.5μ–2.2μ × 3.4μ (membrana excludente) a hyalino pallide brunneis in massa; stipibus graphium 100μ–700μ altis, 15μ–70μ diam., saepe e hypha singula oriundis, pallide brunneis vel hyalinis; conidiis ramis penicillatis mycelii vel synnematibus distinctis enatis, hyalinis, 2μ–3.5μ × 5μ–10μ.

Mycelium hyaline at first, appressed to substratum, becoming light gray-green in 4 to 5 days, never very dark; conidial growth at first almost invisible, graphia soon forming; perithecia slow to form, often absent, black, nearly smooth, spherical, 90μ to 150μ in diameter, necks 200μ to 420μ long, almost cylindrical 30μ to 45μ at base to 25μ to 40μ at ostiole, ostiolar filaments bristlelike, 25μ to 35μ long, pointed; ascospores covered with a gelatinous sheath, kidney-shaped, 1.5μ to 2.2μ by 3.4μ, not including sheath, hyaline to light brown in mass; stalks of graphia 100μ to 700μ high by 15μ to 70μ thick, often originating from single hypha, light brown or hyaline; conidia borne on brushlike branches of the mycelium or on distinct synnema, hyaline, 2μ to 3.5μ by 5μ to 10μ.

Rate of growth, 17 mm in 5 days.

Isolated from pine log (59046 type) at Bogalusa, La., in June 1932, and at Caryville, Fla., (59047) June 1932.

This species was isolated only twice and probably is of little importance as a staining fungus, but it is of interest because of the *Graphium*-like imperfect stage, which is somewhat similar to that of *Ceratostomella ips*. Perithecia are seldom found. None were ever observed in single ascospore cultures, which formed graphia readily.

Ceratostomella pini Münch

Rate of growth, 25 mm in 5 days.

The writer has not made a detailed study of this species, but the culture isolated at Laurel, Miss., fits the descriptions given for it. This isolation has a very dense, conspicuous, white growth of conidiophores over the surface, in which respect it is similar to what Rumbold (*15*) describes as the "eastern strain" of *C. pini*.

Ceratostomella pluriannulata Hedgc.

Rate of growth, 12 mm in 5 days.

This species usually occurs on hardwood logs and lumber, but was occasionally isolated from pine wood. Hedgcock (*7*) first described this fungus, and it was later found in Europe. The manner in which it differs from *Ceratostomella pilifera* and *C. multiannulata* is covered in the discussions of those two species.

Endoconidiophora coerulescens Münch (fig. 2, *D*)

Rate of growth, 33 mm in 5 days.

The microscopical and macroscopical characters of this fungus are identical with those of *Endoconidiophora coerulescens* as described by Lagerberg, Lundberg, and Melin (*9*). In the southern United States it was isolated only from hardwoods, while in Europe it is reported only on spruce and pine. This difference in substratum indicates that there may be a varietal difference between the European and American species. However, a fungus recently collected (in 1934) on *Pseudotsuga taxifolia* Brit. by H. E. Parks in Humboldt County, Calif., has been identified by the writer as *E. coerulescens*. This

indicates that it does occur on some coniferous hosts in North America. Another recent isolation by the writer from yellow poplar

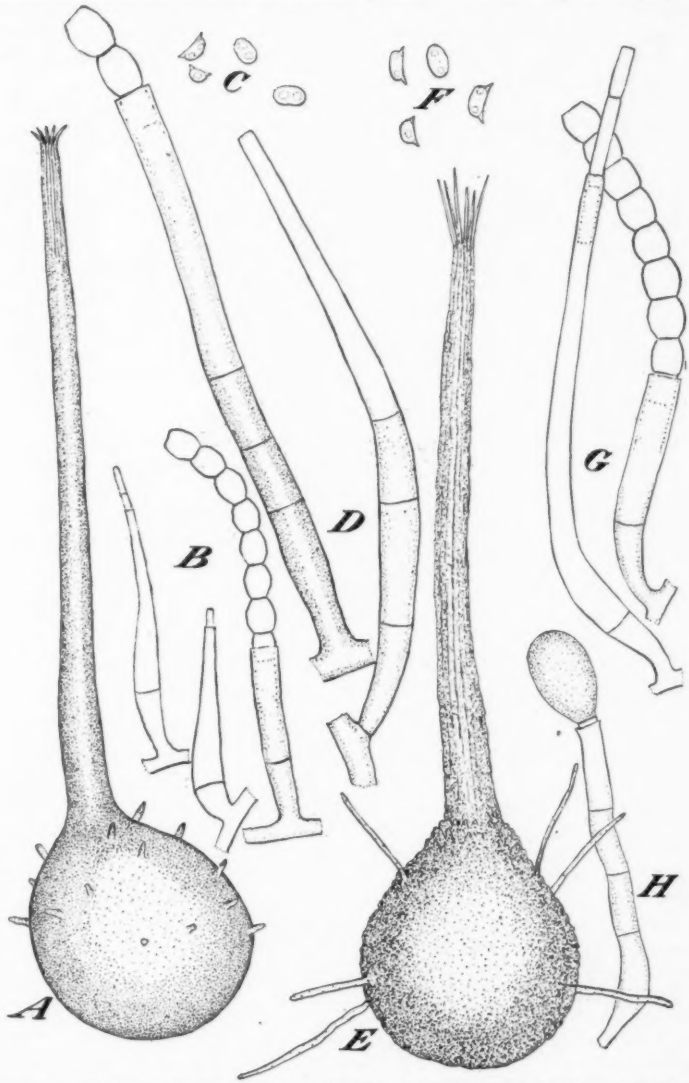

FIGURE 2.—*A–C, Endoconidiophora moniliformis* (Hedgc.): *A*, Perithecium (× 220); *B*, conidiophores and endoconidia (× 1,000); *C*, ascospores in gelatinous sheath (× 1,000). *D*, Conidiophores of *Endoconidiophora coerulescens* Münch (× 1,000). *E–H, E. fimbriata* (Ell. and Hals.); *E*, Mature perithecium (× 220); *F*, ascospores (× 1,000); *G*, conidiophores and endoconidia (× 1,000); *H*, conidiophore and brown endoconidium (× 1,000).

collected in Clarendon, Va., also proves it to be more widespread in this country than the earlier study would indicate.

This species produces a very characteristic and penetrating odor both in culture and in the lumber yard. This odor differs noticeably from that produced by any of the other *Endoconidiophora* species with which the writer has worked. Lagerberg, Lundberg, and Melin (*9*) state that the Swedish *E. coerulescens* has an odor which is due to the production of amyl acetate.

Endoconidiophora moniliformis (Hedgc.), n. comb. (fig. 2, *A–C*)

Ceratostomella moniliformis Hedgc., 1906, Mo. Bot. Gard. Ann. Rept. 17: 59–114.

Mycelium remains hyaline for 5 to 6 days, then gradually becomes light brown; odor similar to banana oil; perithecia form in 1 to 2 days and mature in 2 to 3 days, black, often elongate and lying on their sides with beaks projecting upward at an angle; 170μ to 260μ high by 150μ to 210μ broad, covered with brown bristles 3μ to 3.5μ thick by 18μ to 60μ long, necks black, 550μ to 1,000μ long, 30μ to 36μ thick at the base to 14μ or 15μ at apex, ostiole surrounded by a few (8–12) hyaline filaments 15μ to 25μ long; ascospores broadly ovoid 2μ to 2.8μ by 4μ to 5μ; endoconidiophores of two kinds, one long and attenuated to small diameter (2.5μ to 3μ) at apex and producing narrow hyaline cylindric endoconidia 2.5μ to 3μ by 6μ to 10μ, the other shorter and enlarged slightly above (4.5μ to 6μ), producing hyaline, short, thick, barrel-shaped conidia, 4.5μ to 6μ by 5μ to 7μ, in long moniliform chains.

Rate of growth, 38 mm in 5 days.

This species was the most common one found on hardwood logs and lumber, but produces a less conspicuous stain than *Endoconidiophora coerulescens* because its mycelium is hyaline or very light brown. It may be identified by the pear-shaped perithecia, which are often in a partially reclining position. The perithecia, unlike those of *E. coerulescens*, appear almost smooth under the band lens.

Endoconidiophora fimbriata (Ell. and Hals.), n. comb. (fig. 2, *E–H*)

Ceratocystis fimbriata Ell. and Hals., 1890, N. J. Agr. Expt. Sta. Bull. 76: 14.

Sphaeronema fimbriatum (Ell. and Hals.) Sacc., 1892, Syll. 10: 215.

Ceratostomella fimbriata (Ell. and Hals.) Elliott, 1923, (Abstract) Phytopathology 13: 56.

Rate of growth, 11 mm in 5 days.

This fungus was isolated only once from a brown-stained beech board and is probably of little importance as a stainer of lumber. It is of interest, however, in that it brings to three the number of species of this genus found inhabiting hardwood lumber in the southern United States.

As in *Endoconidiophora coerulescens* (fig. 2, *D*) and *E. moniliformis* (fig. 2, *B*), this strain of *E. fimbriata* has two distinct types of endoconidia (fig. 2, *G*). In all three species one type is narrow and cylindrical in shape and the other barrel-shaped. Conidia of the second type have been observed, however, only in the strain of *E. fimbriata* obtained from wood.

A third type of endogenously formed spore has been commonly described for the sweetpotato strains of *Endoconidiophora fimbriata*, as illustrated by Andrus and Harter (*1, figs. C and D*). Only a few of these spores (fig. 2, *H*) were produced by the strain obtained from lumber. Unlike the first two types of conidia, this type is brown and at maturity is considerably larger in diameter than the conidiophore on which it is produced. This type of conidial formation is more nearly approached in *E. adiposa* (fig. 3, *B* and *D*) and *E. para-*

doxa (fig. 3, *H*), which are also dark-spored species. This spore form has not been observed on *E. coerulescens* or *E. moniliformis*.

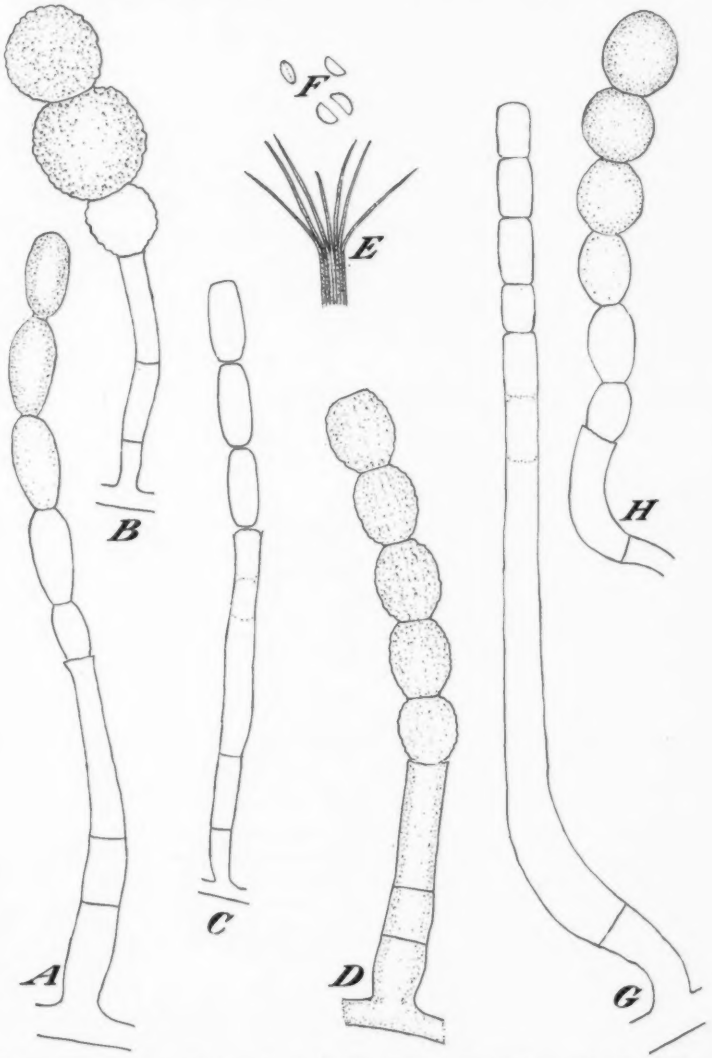

FIGURE 3.—*A-F, Endoconidiophora adiposa* (Butler): *A* and *B*, Conidiophores and endoconidia from culture isolated from *Eleocharis* (× 1,000); *C* and *D*, conidiophores and ends cononidia from culture isoloted from basket veneer (× 1,000); *E*, tip of perithecial beak (× 220); *F*, ascospores (× 1,000); *G* and *H*, *Endoconidiophora paradoxa* (de Seynes), conidiophores and endoconidia (× 1,000).

The following two species were not obtained in this investigation but are included to complete the group of endoconidial species.

Endoconidiophora adiposa (Butler), n. comb. (fig. 3, *A–F*)

Sphaeronema adiposum Butler, 1906, India Dept. Agr. Mem., Bot. Ser., 1, (3), 53 pp.

Ceratostomella adiposum (Butler) Sartoris, 1927, Jour. Agr. Research 35: 577–585.

Rate of growth, 50+mm in 5 days.

This species has been isolated from moldy basket veneer in Baltimore, Md., by Hedgcock, and from *Eleocharis tuberosa* (Roxb.) Schult., from China. The dark endoconidia are produced in great abundance. They vary in surface echinulation from the large spiny ones illustrated by Sartoris (*16, fig. 1, E*) to the slightly roughened ones shown herein (fig. 3, *B* and *D*). Also, great numbers of smooth spores are produced, most of which are hyaline (fig. 3, *C*). These conidia are not exactly comparable to the two types of hyaline conidia produced by *Endoconidiophora coerulescens* and *E. moniliformis*, although they are similar in some respects. Butler (*2*) has thoroughly described this considerable variation in endoconidia of *E. adiposa*. Such great variation has not been found in the endoconidia of the other species studied.

Endoconidiophora paradoxa (De Seynes), n. comb. (fig. 3, *G* and *H*)

Sporoschisma paradoxum De Seynes, 1874, Recherches Végét. Inf. 111, p. 30.

Thielaviopsis paradoxa (De Seynes) Höhn, 1904, Hedw., 43: 295.

Ceratostomella paradoxa (De Seynes) Dade, 1928, Brit. Mycol. Soc. Trans. 13: 184–194.

Rate of growth, 45mm in 5 days.

The conidial form of this species was obtained by the writer along with *E. adiposa* from Chinese *Eleocharis tuberosa*. Spores of the hyaline cylindric type (fig. 3, *G*) were produced in great abundance, as were also the smooth, dark conidia (fig. 3, *H*). The latter are more nearly like those of *E. adiposa* in the way in which they are formed, but the hyaline ones are very similar to the hyaline cylindric spores produced by *E. coerulescens*, *E. moniliformis*, and *E. fimbriata*.

FUNGI IMPERFECTI

A number of additional fungi which may cause some staining of wood were occasionally isolated. They belong for the most part to unrelated groups of the Fungi Imperfecti. *Diplodia* was found to be the most common genus present in freshly stained lumber and logs. *Botryosphaeria ribis* Gross and Dug., which is very similar to *Diplodia natalensis* in culture, was obtained a number of times from somewhat weathered boards, but was never found in freshly stained lumber. *Hormodendron cladosporioides* (Fres.) Sacc. was not obtained from stained wood during this investigation, although it is described by Lagerberg, Lundberg, and Melin (*9*) and by Hedgcock (*7*) as a common staining fungus.

The other imperfect forms, with the possible exception of *Graphium rigidum*, were not important as staining fungi on newly sawed lumber in the localities where this study was made. Some of them, such as *Cadophora*, may stain wood when present, but they seldom appeared in culture during the investigation. Difficulty was experienced in classifying some of these forms from descriptions. In the case of *Cadophora* it is not definitely known whether the species given herein are the same as those described by Kress et al. (*8*). *Cadophora brunnescens* is probably the same species as the one they illustrated

(*8, pl. 20, figs. 3 and 4*). Another fungus they illustrated (*8, pl. 20, fig. 2*) is probably another species of *Cadophora* which differs from either of the species described here.

Diplodia natalensis P. Evans
Rate of growth, 45 mm in 5 days.

Diplodia natalensis grows more rapidly in culture than any other fungus isolated from stained wood. The mycelium is white and fluffy at first, but the aerial mycelium soon becomes dark gray and the substratum black. Sclerotia containing pycnidia and spores usually form in older test-tube cultures when grown on 2½ percent malt agar, but fruits are more readily produced in flasks or test tubes of corn-meal mush.

This species is no doubt one of the important blue-staining fungi occurring in the South. It was always associated with stain in the samples of lumber from which it was isolated.

Diplodia megalospora Berk. and Curt.
Sphaeropsis ellisii Sacc., 1864, Syll. Fung. 3: 300.
Rate of growth, 45 mm in 5 days.

This species differs from *Diplodia natalensis* in having larger spores. It is a common fungus and is known to stain the stems of small infected pine trees a dark blue-gray.

Cadophora brunnescens, n. sp. (fig. 4, *E* and *F*)

Mycelio primum hyalino, dein pallide brunnescente, demum obscure brunneo, mycelio aerophilo brunneo, recte caespitoso, hyphis obscure brunneis, septatis, 2.5μ–4μ diam., plerumque in fasciculis flagelliformibus dispositis; conidiophoris numerosissimis, uniformiter super fasciculos et hyphas singulas distributis, brevibus, 10μ–25μ longis, e basi 2.5μ–4μ ad apicem 1.2μ–2μ diam. attenuatis, apice pallide brunneo collari munitis; conidiis endogenis, primum hyalinis et ovoideis cylindricisve, 1.2μ–3μ × 3μ–8μ, dein globosis, pallide brunneis, 1.5μ–3μ diam.

Mycelium, at first hyaline, becoming light brown in 3 days, then dark brown, aerial mycelium brown, erect tufted, hyphae dark brown, septate 2.5μ to 4μ in diameter, usually in whiplike strands; conidiophores very numerous, uniform over whiplike strands and on individual hyphae, short, 10μ to 25μ long by 2.5μ to 4μ thick at base and tapering to 1.2μ to 2μ above with light-brown flaring collar at apex; conidia formed endogenously, hyaline and ovoid or cylindric at first, 1.2μ to 3μ by 3μ to 8μ, later globose and light brown 1.5μ to 3μ in diameter.

Rate of growth, 5.5 mm in 5 days.

Isolated from pine log (59044 type) at Caryville, Fla., June 1932, and from old red gum board (59048), Urania, La., June 1931.

This species is somewhat similar to *Cadophora fastigiata* Lagerberg and Melin, but it is brown, whereas *C. fastigiata* is dark gray to black, and its conidia are more variable in size and shape than those of *C. fastigiata*.

Cadophora repens, n. sp. (fig. 4, *D*)

Mycelio in agaro (malt) primum hyalino, dein pallide brunnescente, demum obscure brunneo, ad substratum appresso, raris cirrhis aerophilis exceptis, hyphis brunneis, 2μ–4.5μ diam., saepe in fasciculis dispositis; conidiophoris plerumque in ramis brevibus caespitulosis, parvis, 10μ–15μ longis, e basi 2μ–3μ ad apicem 1μ–1.5μ attenuatis, hyalinis vel pallide brunneis; conidiis endogenis, hyalinis, cylindricis, interdum curvatis, 1μ–1.5μ × 2.5μ–8μ.

Mycelium, on malt agar, hyaline at first, becoming light brown in 5 days and finally dark brown, appressed to substratum except for a few aerial tufts of hyphae; hyphae brown 2μ to 4.5μ in diameter, often in strands; conidiophores usually grouped on short branches, small, 10μ to 15μ long, tapering from 2μ to 3μ at base to 1μ to 1.5μ at apex, hyaline or light brown; conidia endogenously formed, hyaline, cylindric, sometimes curved, 1μ to 1.5μ by 2.5μ to 8μ.

Rate of growth, 22 mm in 5 days.

Isolated from pine log (59045 type), Caryville, Fla., June 1932.

This species is distinct because of its very rapid growth in culture and the grouped conidiophores. The conidiophores are usually

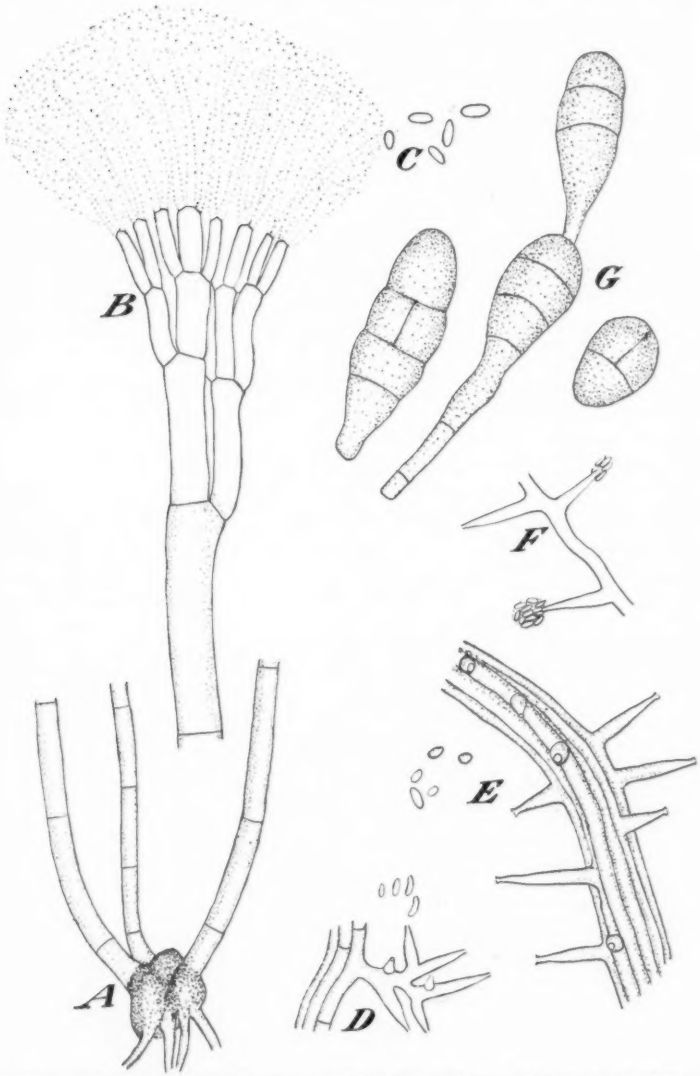

FIGURE 4.—*A–C, Leptographium microsporum: A,* Basal portion of group of conidiophores (× 220). *B,* apex of sporulation conidiophore (× 1,000). *C,* conidia (× 1,000). *D,* Conidiophores and conidia of *Cadophora repens* (× 1,000). *E* and *F, Cadophora brunnescens: E,* Ropelike strand bearing conidiophores (× 1,000); *F,* single hyaline hyphae bearing conidiophores (× 1,000). *G,* Conidia of *Alternaria* sp. (× 1,000).

hyaline or very light brown at the base and have no distinct flaring collar at the apex. The spores are always hyaline.

Leptographium microsporum, n. sp. (fig. 4, *A–C*)

Mycelio primum hyalino, byssoideo, demum obscure griseo et densissime intertexto, conidiophoris tenuibus dense tectis; conidiophoris longe pedicellatis, 5μ–10μ diam., 125μ–500μ longis, apice e capitulo penicillato praeditis, ramis terminalibus hyalinis, apice conidia muco-conglutinata gerentibus; conidiis parvis, 0.9μ–1.5μ × 2.5μ–3.5μ, hyalinis, interdum leniter curvatis.

Mycelium hyaline, cottony at first, finally dark gray and densely matted, covered with dense formation of fine conidiophores; conidiophores long-stalked, 5μ to 10μ in diameter by 125μ to 500μ long, with a brushlike head at apex, terminal branches hyaline and bearing conidia at their apex which cling together in a mucous mass; conidia small, 0.9μ to 1.5μ by 2.5μ to 3.5μ, hyaline, sometimes slightly curved.

Rate of growth, 7.5 mm in 5 days.

Isolated from beech board (59043 type) at Laurel, Miss., May 1932, and from red gum board (59050) at Bogalusa, La., June 1931.

The conidiophores of this fungus are similar to those of *Leptographium penicillatum* Grosmann (*6*) in manner of branching, but there are usually 2 to 5 formed together at rhizomelike nodes as in *Scopularia venusta* Preuss (*10, p. 745*).

The boards from which this fungus was obtained had previously been stained by *Endoconidiophora coerulescens* and also contained *Graphium rigidum*.

Hormonema pullulans (De Bary) Lagerberg and Melin

Hormiscium gelatinosum Hedgc., 1906, Mo. Bot. Gard. Ann. Rept. 17: 59.

Rate of growth, 11 mm in 5 days.

This fungus more nearly fits the description given by Robak (*13*) for *Hormonema pullulans*. Cultures isolated by the writer formed no aerial mycelium but remained entirely smooth on the surface of the substratum.

This species seemed to cause little stain in the fresh lumber from which it was isolated. It was found in slightly stained old boards and may be the cause of considerable staining in such material. It was, of course, difficult to determine definitely just which or how many fungi were responsible for this latter stain.

Helminthosporium geniculatum Tracy and Earle

Rate of growth, 9 mm in 5 days.

This is a slow-growing fungus which was obtained from pine boards placed in the yard for only a few days. It is possible that infection occurred when sample boards were dropped on the grass that grew at the sides of the lumber piles. Species of *Ceratostomella* were always present in the boards from which it was obtained.

This fungus is characterized by its curved brown spores and cylindric sclerotia, both of which are produced in culture.

Graphium rigidum (Pers.) Sacc.

Rate of growth, 12 mm in 5 days.

This species of *Graphium* is very common on hardwood logs and lumber, where it is usually associated with *Ceratostomella pluriannulata* and *Endoconidiophora coerulescens*. In culture the mycelium is very similar in appearance to *C. pluriannulata*, but it fruits readily, so was not confused with that species.

Alternaria sp. (fig. 4, *G*)

Rate of growth, 14 mm in 5 days.

White fluffy mycelium at first, becoming mouse-gray and the substratum dark gray-black; conidiophores form on the older mycelium, usually on or near the substratum, dark brown, 3μ to 4.5μ thick, long or short, septate; spores varying

greatly in shape and size, 8μ to 15μ by 16μ to 35μ, dark brown muriform. Usually 3 or 4 cross walls with one longitudinal septum through the larger part of the spore.

At Laurel, Miss., this fungus was frequently one of the first to be cultured from the boards, but it could never be definitely associated with stain.

SUMMARY

A study made in several localities of the southern United States showed that species of Ceratostomataceae were responsible for most of the initial blue stain in lumber and logs. *Ceratostomella ips* and *C. pilifera* were most frequently isolated from recently stained pine logs and lumber, and *Endoconidiophora coerulescens*, *E. moniliformis*, and *Ceratostomella pluriannulata* from stained hardwood logs and lumber.

A number of Fungi Imperfecti were also obtained from stained lumber, but *Diplodia natalensis* and *Graphium rigidum* were the only ones of this group which seemed to be of considerable importance.

Two new species of *Ceratostomella* were obtained from pine. One of these, *C. multiannulata*, was the most common species found fruiting on lumber but seemed to cause little discoloration in the interior of the wood. *C. obscura*, the other new species, was obtained only twice from stained pine logs.

The genus *Endoconidiophora* has been retained for those species of *Ceratostomella* having endoconidia. Three species were isolated from lumber and two others were studied and placed in this genus.

Three Fungi Imperfecti, *Cadophora brunnescens*, *C. repens*, and *Leptographium microsporum*, obtained from stained wood, are described as new species. They do not seem to be of much importance as wood-staining species.

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EFFECT OF ANNUAL GRASS FIRES ON ORGANIC MATTER AND OTHER CONSTITUENTS OF VIRGIN LONGLEAF PINE SOILS [1]

By S. W. GREENE [2]

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INTRODUCTION

The winter burning of dead grass left unconsumed by grazing animals from the growth of the previous season is a world-wide practice of very ancient origin, particularly in humid regions where uncut grass does not cure into palatable winter forage. By the use of grass fires the Indians maintained open grazing lands for the bison and pronghorn antelope, the largest herds of grazing animals that the world has known. This practice of the Indians was considered beneficial to the land and was continued by white men, particularly in the humid longleaf pine region of the South, as a means of keeping down the underbrush and improving the pasturage for cattle. In the longleaf pine region a large part of the virgin soil is burned over each year. Hilgard (*10, p. 495*),[3] writing of the longleaf area of southern Mississippi, stated:

"The land * * * affords but indifferent pasturage, except the first season after burning-over; probably because of the effect of the minute amount of ashes so added."

Referring to the effect of grass fires on forest growth, Harper (*8, p. 668*) in 1913 stated:

> * * * they return immediately to the soil the mineral plant food stored up in the leaves. The amount of the available plant food in the soil of the pine forests is usually rather limited, and these frequent fires thus enable the pine to do business on a small amount of capital, as it were.

The literature does not seem to record any conflict with the empirical observations of the users of grass fires until recent years, when objections to the use of fires were made on the theoretical basis that the fires destroy organic matter and nitrogen to the detriment of soil fertility. The general unsupported argument against the use of fire was stated by Van Hise (*24, p. 238*) in 1910, as follows:

> The fires do not simply confine themselves to the timber, but they burn the humus in the soil itself. Frequently, after a great forest fire, and especially if the fires run over the same area two or three times, there is left of the soil, sand, and other minerals, but little of the original organic material.

Mattoon (*16, p. 48*) in 1931, states:

> The leaves, or "straw", from pines contain considerable nitrogen and small amounts of phosphoric acid and potash. A ton might contain these essential fertilizing elements to the value of $2 to $4. An unburned pine woods may have as much as 10 to 15 tons [per acre] of leaves and other organic matter.

[1] Received for publication Feb. 18, 1935; issued July, 1935. The data reported were obtained in a cooperative grazing and reforestation experiment conducted at the McNeill Experiment Station, McNeill, Miss., by the Bureaus of Animal Industry and Plant Industry and the Forest Service of the U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station.

[2] The writer is indebted to H. R. Reed, formerly of the Bureau of Plant Industry, who spent considerable time in making the final legume counts. Acknowledgment is made also to W. F. Hand, State chemist of Mississippi, for analyses of soils and for determinations of the protein and ash content of grasses.

[3] Reference is made by number (italic) to Literature Cited, p. 821.

However, Mattoon gives no basis for his statements and does not indicate any way in which organic matter and nitrogen, on top of the virgin soil, might be recovered in the soil for the use of growing plants.

RELATED INVESTIGATIONS

Alway and Rost (*2*), from experimental work in 1918–19, found that burning did not influence the immediate fertility of the mineral soil and concluded that any loss in productivity would depend on the loss of nitrogen contained in the forest litter, since the mineral elements are returned immediately to the soil by fire. In later experiments Alway found that burning the forest floor as compared with plowing under the natural litter had no significant effect on crop production either immediately following (*1*) or over a series of years.[4]

Greene (*7*), in 1929, showed that cattle made 44.4 percent greater agins on burned native grass pastures than on similar unburned pastures over a period of 4 years.

The literature in regard to the accumulation of humus and nitrogen in cultivated soils and their effect on crop production is voluminous. Such literature, however, is concerned exclusively with the problem of green manuring, or the turning under of plant residues, and does not consider the problem of humus and nitrogen or their accumulation in soils that have not been plowed and are to be handled as virgin soils in forests or pastures where vegetation is either burned or left to decay where it accumulates on top of the soil.

The emphasis placed by popular agricultural literature on the value of organic matter plowed under, without regard to the quality or quantity of the material to be turned under, has, no doubt, created a tendency to overestimate the fertilizing value of vegetative growth on virgin soils. Moreover, the theoretical grounds on which deductions may be based are not always realized in practice. Such a concept for virgin soils leaves out of consideration the methods by which nature incorporates plant residues with the soil.

The importance of organic matter in the soil has always been recognized, but a sharp distinction must be drawn between organic matter in the soil and on top of the soil. According to Pieters (*19*), organic matter on top of virgin soils may be incorporated with the soil by the action of water or glaciers, by rodents, insects, earthworms, and micro-organisms, and by the sharp hoofs of grazing animals, but chiefly by the decay of plant roots.

Soil nitrogen in any form is derived originally from the gaseous nitrogen in the air and is a rather unstable and transient material, the gains and losses of which are perhaps not yet fully explained. Small quantities of gaseous nitrogen, in forms available to plants, are added to the soil by rainfall and by free-living micro-organisms, but the chief source of increase is through the action of the micro-organisms associated with legumes. Finnell and Houghton (*6*) found that 15.45 inches of rainfall in 1930 added 1.42 pounds of nitrogen per acre. No measure of the quantity of nitrogen per acre fixed by free-living organisms seems to have been obtained, and the effect of this class of organisms appears to be almost purely speculative.

[4] Unpublished information.

Nitrogen is an essential element of plant growth, and the nitrogen content of the soil is closely associated with the organic-matter content. Plants other than legumes, grown and left in place, do not add nitrogen to the soil but transform soil nitrogen into organic nitrogen with a loss to the soil in the process. Because of the known ability of legumes to store a considerable quantity of nitrogen extracted from the air, in both the root and aboveground portions of the plant, the maintenance or increase of nitrogen in virgin soils may depend largely on the character of the plant population.

In annual legumes one-fourth to one-third of the total nitrogen has been found by the Mississippi Agricultural Experiment Station to be in the portion of the plants below ground,[5] and in some perennial legumes nearly half of the nitrogen is in the underground portions (*19, p. 74*).

Lyon and Bizzell (*15*), by lysimeter experiments, for a period of 15 years, in which the nitrogen added by rainfall and manure and removed by the crops and in the drainage water was carefully measured, found that in tanks containing growing legumes there was an increase of nitrogen in the soil equivalent to about 60 pounds per acre per year. When the tanks did not produce crops for 10 years and then grew nonlegumes for 5 years, the loss of nitrogen from the soil was at the rate of about 25 pounds per acre per year.

Sievers and Holtz (*21*) have shown that both the organic-matter and nitrogen increase in soils is influenced by the nitrogen-carbon ratio of the plants grown on the soil or turned under.

In the mature stages of plants it has been shown by LeClerc and Breazeale (*12*) and others that leaching of the mineral elements from plants in place occurs in considerable quantities, but that leaching of nitrogen is relatively unimportant.

Soil nitrogen is decreased by the removal of crops which contain nitrogen, by conversion into ammonia gas and gaseous nitrogen through the action of micro-organisms, both bacteria and fungi, and by leaching.

Heck, Musbach, and Whitson, as reported by Clark (*4*), found that the loss of organic nitrogen from manures on the ground in free circulation of air is much more rapid than below ground. The same relation between nitrogen in the aboveground and below-ground portions of mature plants would be indicated.

Nitrate nitrogen, the form most available as a plant nutrient, is readily soluble and is quickly leached away if not taken up by growing plants. Lyon and Bizzell (*14*) have shown that the loss of nitrogen by leaching was 17 times as much on uncropped land as on cropped land. Because of the loss of nitrogen both as ammonia and as nitrate on uncropped land, the use of winter cover crops as well as summer growing crops is generally advocated to conserve nitrogen on tilled soils in humid areas with mild winters. On virgin soils a constant maximum growth of herbage is indicated to conserve soil nitrogen.

The foregoing references underlie the concept derived from the experimental data to be presented. Although fire has, no doubt, been the most violent if not the most active chemical reaction present on the soil of the virgin longleaf pine region and is known to have a very active influence on the plant population of virgin soils, no data other

[5] Unpublished information.

than the limited work cited have been found showing the influence of frequent or annual burning on the fertility of the soil, as compared with protection from fire.

EXPERIMENTAL PROCEDURE

A tract of 320 acres of virgin land near McNeill, Miss., reproducing to longleaf pine about 20 years after the virgin timber had been removed, was fenced and divided into four experimental areas in 1923. Previously the land had been unfenced open range, subject to annual or periodic burning and lightly grazed by cattle. Since the fenced area was a part of a large area of open land with no natural barriers to fire or grazing, it is believed that the areas as divided had received uniform treatment before being fenced. This area is typical of the rolling longleaf pine hills of southern Mississippi. It has an elevation of 230 feet, and is well drained, as are the soils of the heavier type in longleaf pine areas.

Two areas of 150 acres each were grazed, one of which was burned annually during winter or early spring, beginning in 1923. Two 10-acre areas were not grazed, and one of these was burned annually. A detailed soil map of the area, made by the Bureau of Chemistry and Soils of the United States Department of Agriculture, was used as an aid in establishing the experimental areas and plots.

No soil analyses were made at the beginning of the experiment, since the studies undertaken concerned mostly problems of forage production. Soil analyses were made later in seeking an explanation of differences in forage growth under different treatments. Detailed studies of the variations in the plant population were made annually.

To determine the effect, on the soil, of burning the grass annually over a considerable period of time, analyses were made of soil samples collected on April 23, 1929, about 3 months after growth had begun in the spring and during the season of flush growth, and on January 10, 1930, during the dormant period. As it was recognized that individual samples might vary in chemical composition within a radius of a few feet, five random samples were taken to a depth of 6 inches for each soil type, and these five were mixed thoroughly to form a composite sample.[6] In addition to the principal studies relating to organic matter and nitrogen content of the soil, other tests dealt with density of plant growth, moisture content, and micro-organisms in soil from burned and unburned areas.

EXPERIMENTAL RESULTS

ORGANIC MATTER AND NITROGEN

Table 1 shows the content of organic matter and nitrogen from moisture-free samples of soil on the dates mentioned.

In samples taken in April the average organic-matter content of soil from the burned areas was 3.17 percent as compared with 2.59 percent for the unburned areas, or a ratio of about 1.2:1. The corresponding figures for nitrogen content were 0.047 and 0.055, or a ratio of about 0.9:1. Although the samples collected in April showed a variation in organic matter in favor of the burned areas in each instance, the proportion of nitrogen to organic matter was sometimes

[6] Soil samples were collected by the Southern Forest Experiment Station of the U. S. Forest Service. Soil analyses were made by W. F. Hand, State chemist of Mississippi.

TABLE 1.—*Organic matter* [1] *and nitrogen in composite samples of moisture-free soils taken Apr. 23, 1929, and Jan. 10, 1930, from soils subjected for 7 and 8 years, respectively, to the treatment indicated*

Soil type [2] (fine sandy loam)	Grazed area				Ungrazed area			
	Burned		Unburned		Burned		Unburned	
	Organic matter	Nitrogen	Organic matter	Nitrogen	Organic matter	Nitrogen	Organic matter	Nitrogen
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
APRIL 23, 1929, SAMPLES								
Orangeburg	2.41	0.033	2.30	0.050	2.47	0.045	2.33	0.040
Norfolk	3.36	.046	2.62	.050	2.67	.050	[3]	[3]
Ruston	4.81	.053	2.40	.050	[3]	[3]	2.98	.050
Kalmia	3.29	.053	2.89	.090	[3]	[3]	[3]	[3]
Average	3.47	.046	2.55	.060	2.57	.047	2.65	.045
JANUARY 10, 1930, SAMPLES								
Orangeburg	3.62	0.06	2.26	0.04	4.21	0.07	2.85	0.05
Norfolk	3.87	.06	2.87	.05	3.88	.07	[3]	[3]
Ruston	3.47	.07	2.41	.04	[3]	[3]	2.24	.04
Kalmia	6.90	.10	3.15	.07	[3]	[3]	[3]	[3]
Average	4.46	.07	2.67	.05	4.05	.07	2.54	.04

[1] Analysis made by ignition method.
[2] For description see soil survey by Smith and Carter (*22*).
[3] No sample taken.

in favor of the burned areas and sometimes in favor of the unburned areas. It is known that the available nitrate nitrogen is taken up rapidly in the early flush of forage growth. A simple calculation from the known yields and analyses of the forage shows that the difference in nitrogen content for different areas, after the growing season had advanced approximately 90 days, may have been sufficient to be reflected in the analyses of the soil.

The soil samples taken January 10, 1930, during the dormant period of plant growth for the region, were repetitions of samples taken April 23, 1929, during the season of flush growth of the native vegetation of the region and after about 3 months of the growing season.

In the soil samples taken in January, the variation in organic matter and nitrogen from burned and unburned areas during the dormant period for plant growth, as shown in table 1, was reasonably constant. The average content of organic matter in all samples from burned areas was 4.32 percent as compared with 2.63 percent for the unburned areas, or a ratio of about 1.6:1. The corresponding average differences in nitrogen content were 0.072 percent and 0.048 percent, or a ratio of 1.5:1. The percentages of organic matter and nitrogen were in all cases higher on the burned areas. The highest percentage of organic matter from any unburned sample did not equal the lowest from any burned sample.

The differences in January between the burned and unburned areas in both organic matter and nitrogen were of such magnitude as to be significant. A difference in weight of 0.01 percent in a moisture-free sample of soil to a depth of 6 inches would amount to approximately 155 pounds per acre, according to the weights of Mississippi soils as

given by Logan (*13*). In terms of nitrogen for soils that are deficient in this element, a difference of 0.001 percent, when analysis is made to the third decimal point, is significant. The average difference in percentage of nitrogen was 0.024 percent (0.072—0.048), which represents approximately 400 pounds of nitrogen per acre, in favor of burning. This difference is equal in amount to the nitrogen in an application of approximately 2,400 pounds of nitrate of soda, the common nitrogenous fertilizer of the region.

The conclusion seems warranted that sampling soils for nitrogen during the growing season would give unreliable comparisons for soils that are producing crops varying widely in quantity and quality, especially for soils deficient or low in nitrogen, where the current supply of available nitrogen is a limiting factor for plant growth.

With respect to the soil samples taken at a time of year when plant growth is practically dormant, and over a large area which previously had been subject to uniform treatment, it is reasonable to conclude that the differences in soil analyses were due directly or indirectly to differences in the treatment while under control.

GROWTH OF FORAGE

Throughout the course of the experiment, the growth of forage in the different areas was observed annually on a series of more than 50 rectangular plots of 0.01 acre each.

In 1930, after 8 years of annual burning as compared with complete fire protection over the same period, weights were taken of forage on burned and unburned areas where neither had been grazed (table 2). Two of the 0.01-acre plots were used for each of the two predominating grasses, *Andropogon scoparius* (little bluestem) and *A. tener*, where they were growing in practically pure stands, and two plots for mixed stands. These were selected in the open, away from shade and only a few yards apart on each side of the fire line so that all conditions would be the same except the factor of fire.

TABLE 2.—*Yield per acre* [1] *of the predominating grasses on ungrazed areas, burned and unburned, for 8 years*

Kind of grass	Green plant per acre on—	
	Burned area	Unburned area
	Pounds	*Pounds*
Andropogon scoparius	5,121	3,623
A. tener	6,957	1,206
Mixed stand	5,749	2,415
Average	5,942	2,415

[1] The grasses were clipped about one-half inch above the ground.

The differences in weight on the burned and unburned areas were due chiefly to the reduction of stands (smaller number of plants per acre) on the unburned plot, although there was a reduction in the vigor of growth on this plot as shown by the characteristic spindly growth of plants that are required to grow through a mulch that reduces light in the early stages of growth. The average of the maximum heights

of the plants on the plots studied in the burned area was 20 inches. The corresponding figure for the unburned area was 30 inches. From these studies it appeared that the density of the grass growth on areas that were not burned was reduced from year to year by the smothering or mulching effect of the dead debris of the grasses themselves. On unburned areas where the grasses were further blanketed by the "strawfall" from pine saplings, forage growth was almost completely eliminated in the course of a few years. This reduction in forage growth through the action of dead debris on unburned land is cumulative from year to year and is most rapid where the forage is not grazed by cattle.

Laird (*11*), working with five important sod-forming grasses under pasture conditions, found that approximately 50 percent of the dry weight of the plants was in the root system and that most of the weight of the root was in the 8 inches just below the surface of the soil.

On the experimental area 30 species of native legumes,[7] most of which are perennials, occur in considerable abundance. In 1931, after 9 years of burning, counts of the legume plants were made in each of the 0.01-acre plots in the four experimental areas. From these counts the number of legume plants per acre was calculated, as shown in table 3.

TABLE 3.—*Comparison of legume plants on burned and unburned areas*

Treatment of area	Legume plants per acre on—	
	Grazed area	Ungrazed area
	Number	*Number*
Burned annually (9 years)	35,700	41,500
Unburned	27,600	17,600
Difference	8,100	23,900

From tables 2 and 3 it appears that the cumulative smothering effect of unburned plant debris not only reduced the grasses on the protected areas but also reduced the legumes to the extent that after 9 years of fire protection they were less than half as numerous as on the area burned annually over the same period, where neither area had been grazed. These counts confirm observations made in Georgia by Stoddard (*23*) and in Florida by Harper (*9*). In the grazed areas the number of legumes on the protected plots also was materially less though the difference was not so marked.

A strip survey[8] of the forage cover of the entire county in which the experimental area was located showed that the legumes were most abundant on the well-drained soils. This observation confirms those of previous investigators.

The burned and ungrazed area had one more legume plant for approximately each 2 square feet than had the unburned and ungrazed area. The probable effect of this difference in legume population on the quantity of nitrogen gathered from the air is obvious. It is also

[7] The seeds of 20 species found at McNeill, Miss., have been determined by Stoddard (*23*) to be important quail feed.

[8] A sampling process based on studies of representative small plots at regular intervals.

clear that doubling the plant growth per acre would greatly increase the accumulation of organic matter in the soil through decay of plant roots.

CRUDE PROTEIN AND ASH IN FORAGE

It is to be expected that the difference in nitrogen in soils from burned and unburned areas, as shown in table 1, would be reflected somewhat in a difference in crude protein in forage from those areas. The quantity of the protein in plants indicates the fertility of the soil, particularly soil that is deficient in nitrogen. To compare the forage from the burned and unburned areas for content of crude protein, and also for ash, samples of the two predominating species of grasses, *Andropogon scoparius* and *A. tener*, were taken in 1929 and again in 1931 after 7 and 9 years of burning and fire control, respectively. Composite samples were made by cutting and mixing the current growth from a large number of random locations. Samples were taken from April to June during the flush of the early season's growth. Table 4 presents a summary of the results obtained. No sample from the unburned areas equaled the comparable sample from the burned areas.

TABLE 4.—*Comparison of the average crude protein and ash content of moisture-free samples of grasses on burned and unburned areas*

Treatment of area	Crude protein	Ash
	Percent	*Percent*
Burned annually (7 and 9 years)	10.15	7.92
Unburned	7.77	6.86
Difference	2.38	1.06

A difference of 2.38 percent in crude protein is sufficient to affect the feeding value of the forage, and the sale value of commercial feeds is determined largely by the difference in protein content. A difference of 1.06 percent in ash also would affect the value of forage plants as feed (*3*, *20*).

MINERAL FERTILIZING ELEMENTS

In 1930, after 8 years of burning and fire control, soil samples representing the four soil types in the burned and unburned areas were analyzed for their content of mineral elements, namely, aluminum oxide, magnesium oxide, calcium oxide, potash, phosphoric acid, and sulphur trioxide. The total quantity of these minerals in the ash was as follows: Burned areas, average of all samples, 2.581 percent; unburned areas, 1.899 percent; difference in favor of burned areas, 0.682 percent. No sample from the unburned areas equaled the comparable sample from the burned areas.

These analyses of the mineral fertilizing elements of the soil substantiate the statements of previous investigators, already presented, that burning does not deplete the mineral fertility of the soil, but returns it directly to the soil where it becomes quickly available for the growth of plants.

SOIL MOISTURE

As a secondary influence of increased organic matter in the soil, it would be expected that soil moisture would increase because of the known water-holding capacity of organic matter in soil.

The region under consideration has a heavy rainfall distributed throughout every month of the year, November having the minimum amount. The average rainfall in November over a 27-year period was 3 inches. Ordinarily there is no deficiency in surface moisture except on steep slopes or light sandy soils, although during periods of drought the soil moisture is rapidly reduced.

The data obtained on soil moisture were based on samples representing different soil types and varying degrees of vegetative growth. Individual samples collected during the growing season accordingly showed considerable variation with no particular trend. In view of the rather wide variation in organic matter for different soil types and the patchy vegetative growth on the unburned pasture, due to spotted grazing, it was realized that the different conditions might give rise to unreliable comparisons unless large numbers of samples were taken. Accordingly more than a thousand individual samples were obtained to make more than 200 composite samples. These represented soil from the surface to a depth of 12 inches for both the burned and unburned areas.

Each of the composite samples was made up of from 5 to 10 individual samples taken within a radius of about 10 feet. No sample was taken less than 24 hours after a rainfall. The results of this investigation are given in table 5.

TABLE 5.—*Average percentage of soil moisture in samples of soil from burned and unburned areas, Apr. 21–Oct. 9, 1931*

Treatment of area	Composite samples	Moisture
	Number	*Percent*
Unburned	124	9.63
Burned	119	9.46
Difference	5	.17

Table 5 shows no significant difference in the moisture content of the burned and unburned areas. However, in considering the moisture data, the wide variation between plant growth on the burned and unburned areas must be taken into account since a ton crop of hay per acre requires the use of about 250 tons of water during the period of its growth (*18*). When the moisture taken from the soil and transpired by a much larger forage production is considered, the conclusion seems warranted that the additional organic matter in the soils on the burned areas must have increased their water-holding capacity to a considerable extent. This is further confirmed by moisture determinations of surface-soil samples taken in May 1930 during the flush of plant growth, and in December after the plant growth was mature. The popular conception is that a mulch of litter on top of the soil conserves moisture at the surface, and the samples which furnished the data shown in table 6 were taken to a depth of 1 inch.

TABLE 6.—*Soil moisture of burned and unburned areas at a depth of 1 inch*

Condition of area	Moisture content of soil [1] on—		Condition of area	Moisture content of soil [1] on—	
	May 21, 1930	Dec. 6, 1930		May 21, 1930	Dec. 6, 1930
Burned:	*Percent*	*Percent*	Unburned:	*Percent*	*Percent*
Grazed	10.3	11.1	Grazed	9.9	9.9
Ungrazed	9.3	14.9	Ungrazed	10.0	13.2

[1] Rainfall for the 30 days ended May 19 was 3.25 inches and for the 30 days ended Dec. 5, 6.37 inches. Of these quantities 2.09 inches fell on May 19 and 0.55 inch on Dec. 5.

Although the burned and ungrazed area produced a forage growth more than double that on the adjacent unburned and ungrazed area, it still maintained its moisture content and ended the growing season with 1.7 percent more moisture.

Merkle and Irvin (*17*) have shown that laboratory results in the conservation of moisture by a mulch, where the soil tubes are in contact with a water table, do not apply in practice to field conditions where no such water table exists near the surface of the soil. In the latter case, the conclusion seems warranted that the maintenance of soil moisture on the burned areas was due to the increased organic matter in the soil, and that the mulch of accumulated debris on the unburned area did not have the effect popularly described.

Water absorbed by plant debris, in place on top of the soil, is subject to the same sharp division from soil moisture that has already been made between organic matter on top of soil and that incorporated with the soil. It is obvious that any water absorbed by the litter is held away from the soil until it is evaporated. It is obvious also that evaporation is more rapid in the presence of free-air circulation than after moisture is absorbed in the soil. However, the water absorbed directly by the surface litter is a very small percentage of the total rainfall. Samples of a 9-year accumulation of three kinds of plant debris were carefully removed from measured areas on the experimental tract and air-dried. The quantity of water they would absorb to the saturation point was then determined by weight and calculated in terms of inches of rainfall. It was found that the maximum quantity, 0.11 inch, was absorbed by pine straw. Oak leaves absorbed 0.09 inch and dead grass 0.05 inch.

A mulch of plant debris in humid areas appears to affect the soil moisture chiefly by suppressing plant growth, which would take up moisture and transpire it through the leaves. The quantity of water absorbed by such litter is so small that it would not influence soil moisture to any appreciable extent, although it might absorb light showers and deprive surface-feeding plants of a temporary supply of moisture that would freshen plant growth in times of dry weather. Such effects on pastures from showers are well known. It has already been shown that accumulated litter did not raise the soil-moisture content even where it has suppressed plant growth about one-half. Any effect popularly ascribed to a leaf litter, in this case did not compensate for the increased soil moisture held in an adjacent soil with a higher organic-matter content, although the adjacent burned-over soil was supporting about twice the plant growth per acre.

SOIL FLORA

The micro-organisms of the soil are known to be greatly increased by the addition of organic matter, since the organic matter furnishes the energy and nitrogen necessary for growth and reproduction. However, counts of soil organisms vary greatly from time to time and are not a reliable index of soil fertility. The following counts are presented merely as being of interest.

The average bacterial count per gram of soil in 1930 was 1,242,000 for the burned and 857,000 for the unburned areas. In only 1 sample out of 11 did the count on an unburned sample exceed that on the corresponding burned sample. The difference in favor of the burned samples was 385,000 organisms per gram of soil. A larger number of bacteria in the burned soil would be expected in view of the greater amount of organic matter and nitrogen there, as shown by previous analyses. Samples also were collected before and after burning, for the same season. Of 5 sets of samples compared in this way, 3 showed decreases in soil organisms after burning and 2 showed increases. The increases were so great on the two samples, however, that the average increase following the fire was 258,000 organisms per gram of soil.

Coleman (*5*) has shown that the activities of soil organisms are greatest at temperatures between 86° and 100° F. The average of nine soil temperatures taken to a depth of 3 inches between March 16, 1924, and April 24, 1924, after a 3-year accumulation of plant debris on the unburned area, was 78° for the burned area and 72.5° for the unburned area, or a difference of 5.5° in favor of the burned area. It should be understood, of course, that the temperatures were not taken on or near the days of actual burning, and that the higher temperature was the nearer to the range of optimum temperatures. The results tend to account for the average higher bacterial counts following burning.

DISCUSSION

Although the soil and topography in southern Mississippi vary somewhat from those of other locations in the longleaf pine belt, the growth of grasses may be considered in general as typical for the entire area of well-drained lands. In a region more than 1,200 miles in extent, which once grew one species of tree in almost pure stands, it would be expected that factors influencing the growth of associated grasses and legumes on the forest floor would be nearly constant enough to produce in general a rather uniform herbage growth; and this growth is clearly associated with the effects of centuries of periodic grass fires.

To say that burning the organic matter in the form of plant debris on the forest floor or on top of virgin soils tends to increase the organic matter and nitrogen content of the soil may seem paradoxical. Yet it has been shown that the increase of organic matter in the soil is due primarily to the decay of plant roots and that incorporation of plant debris with the soil to form humus is an extremely slow process. It is evident also that any factor that increases the number of plants per acre will of necessity increase the formation of organic matter through the addition of roots to the soil, regardless of what becomes of the tops of the plants. In the experiments described the annual use of winter grass fires approximately doubled the growth of ungrazed grasses and legumes per acre over that produced on similar areas com-

pletely protected from fire, and caused a corresponding increase in the soil organic matter to a depth of 6 inches.

The soils considered are relatively heavy soils for the region. On lighter sandy soil, where the vegetative growth is much less dense, the reduction in plant growth through fire protection and the subsequent effect on the soil would necessarily be a much slower process.

It has been pointed out that nitrogen in the soil is derived from the gaseous form in the air, principally through the medium of bacteria associated with legumes, and that there is a constant loss of soil nitrogen which must be replaced if the soil nitrogen is to be held in balance or increased. In these experiments the annual use of winter grass fires maintained a legume growth on an ungrazed burned area about twice that on a similar area protected from fire, and the hypothesis seems entirely warranted that this increased legume population has caused a corresponding increase of nitrogen on the burned areas over that on the unburned areas. The difference shown by soil analyses may be due, however, partly to an increase of nitrogen on the burned areas and partly to a loss of nitrogen by leaching from the unburned soils where the current plant growth has been greatly reduced.

In studies of the effect of burning on mineral fertilizing elements of the soil, there was a marked difference in favor of the burned areas. Data obtained on soil moisture showed no significant differences, in actual moisture content, but the burned areas produced larger yields of plant growth with attendant larger moisture requirements which evidently were supplied. Data on soil micro-organisms indicate that burning tends to increase their number.

SUMMARY

Analyses of soils taken after 8 years of annual grass burning as compared with complete fire protection on rolling longleaf pine land in southern Mississippi showed 1.6 times as much organic matter in the burned-over soils as in the soils protected from fire. The burned-over soils also contained 1.5 times as much nitrogen as the soils protected from fire. The greater quantities of organic matter and nitrogen apparently result chiefly from roots rather than from tops of plants.

Whether plant debris was burned in place on top of the soil, or was left to rot in place on top of the soil, apparently had no direct effect on either the organic-matter content or the nitrogen content of the soil. In both cases, the organic matter and nitrogen aboveground were largely lost to the soil and the nonvolatile mineral fertilizing elements were returned, leaving organic matter and nitrogen increases to be influenced by the amount and composition of decaying plant roots.

Studies of grass and legume growth on the areas for periods of 8 and 9 years, respectively, showed that the quantity of forage growth on the ungrazed burned areas at the end of the period was more than double that on the unburned areas. The additional quantities of plant roots decaying in the soil on the burned areas apparently account for the increase in soil organic matter to a depth of 6 inches.

The increased growth, on the burned areas, of native legumes, their ability to take nitrogen from the air, and the additional growth of other plants which take up soluble forms of nitrogen and prevent leaching, apparently account for the increased amount of soil nitrogen.

The increase in organic matter and nitrogen on the burned areas was reflected in the higher crude-protein content of the principal forage grasses that grew on burned areas as contrasted with the unburned.

Annual burning returned the nonvolatile fertilizing elements to the soil immediately; this was shown in the analyses of both the soil and the forage growth.

The increased organic matter and nitrogen in the burned-over soils was reflected in an increased number of soil micro-organisms.

The accumulation of plant debris on top of the soil did not materially increase the soil moisture in spite of the fact that much greater amounts of water were required to support the extra forage growth on the burned-over soils.

Organic matter on top of the soil absorbs a portion of the rainfall which is thus prevented from reaching the soil for the use of growing plants.

On the forest floor or on virgin soils, that are not to be plowed, a sharp distinction must be made between the value of organic matter in the soil and organic matter in place on top of the soil.

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HISTOLOGICAL STUDIES OF WISCONSIN HOLLANDER AND WISCONSIN BALLHEAD CABBAGE IN RELATION TO RESISTANCE TO YELLOWS[1]

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INTRODUCTION

The cabbage yellows organism, *Fusarium conglutinans* Wr., was reported as a vascular parasite by Smith (*8*) [2] and later by Harter (*4*), who showed that it formed microconidia in the vessels of the living plant. Tisdale (*11*) found that it penetrated the root hairs of cabbage plants growing on artificial media, and for some time this was accepted as its chief avenue of entrance of the host. Smith and Walker (*9*) found invasion to occur chiefly through the rootcap and the zone of elongation, and only infrequently through root hairs. Penetration was usually intercellular and the fungus made its way into the protostele while that tissue was still undergoing differentiation. The avenue of entrance was the same in susceptible and homozygous resistant lines of cabbage, although penetration seldom occurred in the latter. Resistance to the fungus prevailed throughout the embryonic and permanent tissues of the root, and was attributed to antagonistic chemical substances or physiological qualities of the protoplasm, rather than to morphological characters of the tissues.

In the lines developed by Walker and his associates (*12, 13, 14, 15, 16*) resistance was shown to behave as a single dominant Mendelian factor. In a study of the breeding behavior of individuals of the Wisconsin Hollander variety of cabbage developed by Jones and associates (*5, 6*), Anderson (*1*) showed that resistance in that strain was not necessarily dominant nor was it controlled by a single factor. Phenotypically the resistance of Wisconsin Hollander was intermediate between that of the homozygous resistant lines and the susceptible commercial stocks in that it was commonly effective in the field but tended to break down under relatively high soil temperatures in the field or greenhouse.

The purpose of the present investigation was to study comparatively the host-parasite relationship in a susceptible Hollander strain of cabbage, in the intermediate resistant type of cabbage represented by Wisconsin Hollander, and in a homozygous resistant line developed from the same original commercial type, represented by the Wisconsin Ballhead variety already described by Walker and Blank (*14*).

MATERIALS AND METHODS

Wisconsin Hollander and Wisconsin Ballhead were both selected from the commercial variety known as Hollander or Danish Ballhead.

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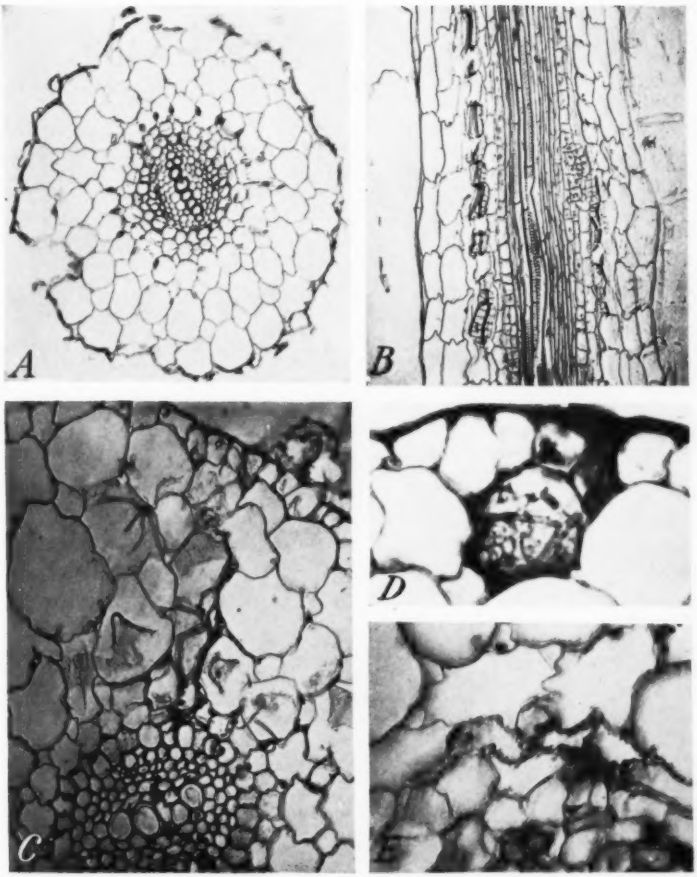
[2] Reference is made by number (italic) to Literature Cited, p. 836.

The Wisconsin Ballhead seed used was lot no. 3331B, described by Walker and Blank (*14*). This was shown by their tests to be homozygous for resistance. Tests by Anderson (*1*) showed it to remain healthy in yellows-infested soil at constant soil temperatures as high as 26° C. Two single-plant progenies secured by Anderson (*1*) from Wisconsin Hollander were selected. One of these, progeny 33s, was very susceptible and will be referred to in this paper as susceptible Hollander; the other, progeny 11s, was one of the most resistant lines derived from this variety and is referred to hereafter as Wisconsin Hollander. It showed a high percentage of resistant plants in the field, but differed from Wisconsin Ballhead in that resistance of the parent plant was not completely dominant, while its progeny from self-pollination succumbed to yellows at soil temperatures of about 24° C.

In order to study different aspects of infection, plants were grown under three types of environment: (1) Seeds placed on artificial media inoculated with the yellows organism; (2) seeds planted directly in inoculated soil; and (3) 30-day-old plants transplanted into inoculated soil. For infection on artificial media, seeds were surface-sterilized in 1 to 1,000 mercuric chloride solution, washed in sterile water, and germinated on potato-dextrose agar. Soil-extract agar in Petri dishes was inoculated in several places across each plate with a single-spore culture of *Fusarium conglutinans*. Seeds in which the radicle was breaking through the seed coat were placed on the inoculated plates slightly in advance of the extending fungus. The culture plates were then placed in a constant temperature chamber at 24° C. and so arranged that the seedlings were held in a normal growing position. Seedlings grown directly in soil inoculated with a cornmeal-sand culture of the fungus were first germinated on potato-dextrose agar and then planted when the radicles were 2 to 3 mm in length. In this series two types of soil treatment were employed: (1) Virgin soil on which no cultivated crops had grown was inoculated directly; (2) greenhouse soil which was steamed under 10 pounds of pressure for 4 hours was later inoculated. The plantings were made in a greenhouse where the soil temperature ranged from 22° to 24°. In another series seedlings were grown for 30 days on soil free from the yellows organism and then transplanted to inoculated soil. The soil temperature was held quite constant at about 22° by the use of Wisconsin soil-temperature tanks.

At various intervals plants were removed for fixation and embedding in paraffin. Plants in soil were carefully removed and the surrounding soil loosened by gentle agitation in water. Even with utmost care plant parts were often missing, especially in severely infected roots. Formol acetic alcohol and Gilson's fixatives were employed, and the staining procedure followed throughout was a combination of Delafield's haematoxylin, safranine, and orange G. In most cases the whole root was cut in serial sections which were 10 to 15 microns in thickness. In the series where 30-day-old plants were transplanted to inoculated soil, sectioning was limited to the region just below the soil level where secondary roots were abundant.



WISCONSIN HOLLANDER ROOTS.

A, Two-day-old root showing suberized thickenings on the radial walls of the cortical cells surrounding the endodermis, × 136; *B*, 3-day-old root showing the thickenings in the same layer of cortical cells, × 70; *C*, 6-day-old root showing cortical infection extending inter- and intracellularly to the pericycle, × 190; *D*, 6-day-old root grown on soil-extract agar, showing penetration of the epidermis and hypodermis accompanied by granulation of the cell contents, × 635; *E*, root of a 6-day-old plant showing suberization of endodermis and pericycle successfully inhibiting fungus invasion of the xylem, × 444.

NORMAL ANATOMY OF THE CABBAGE ROOT

Since the anatomy of the cabbage root has been discussed by earlier writers (*7, 9, 17*) only a brief description of the young root is given. The general structure of the young cabbage root with its diarch protostele and alternate arrangement of xylem and phloem is shown in plate 1, *A* and *B*. A rootcap about six cells in thickness at the root tip decreases to a single layer of cells as it extends back along the sides of the root for a distance of about 1 mm. The epidermis gives rise to the root hairs beginning just above the zone of elongation.

The cortex consists of 4 to 5 layers of cells with large intercellular spaces. The endodermis is rather inconspicuous, but the layer of cells surrounding it is outstanding because of a heavily suberized thickening in each cell. This thickening forms an oval band or ring centrally located on the inner surface of the radial and cross walls. Smaller meshlike thickenings often extend from this main ring, especially centripetally, where they may extend over the inner tangential wall (pl. 1, *A* and *B*). This layer is supportive in nature and often persists when the outer part of the cortex is detached. These peculiar thickenings are not always present and may be totally lacking or interrupted in parts of the same root. Infrequently they occur locally on two rows of cortical cells. The cortical cells enlarge somewhat as root growth continues but soon become loosened and slough off. As the cortex becomes detached, a permanent periderm is formed as a result of tangential division of the pericycle cells. The characteristic root structure persists to the upper region of the hypocotyl.

Tims (*10*) and Smith and Walker (*9*) found no morphological differences in roots of susceptible and resistant plants. No morphological differences were noticed in the present study, but some differences in reaction to the parasite will be discussed later.

COMPARISON OF INFECTION IN SUSCEPTIBLE HOLLANDER, WISCONSIN HOLLANDER, AND WISCONSIN BALLHEAD

Plants of the susceptible and the two resistant strains were exposed to the fungus on agar plates and in inoculated soil. The roots and hypocotyls were fixed, embedded, and sectioned serially. In table 1 is a summary of the results secured from seedlings on agar plates. By the fifth day the root tips and the cortex and stele of both root and hypocotyl of susceptible Hollander were heavily infected. In Wisconsin Hollander (intermediate resistant) and Wisconsin Ballhead (homozygous resistant) invasion of the root tip and of the cortex of root and hypocotyl was common, while invasion of the stele was less pronounced.

TABLE 1.—*Occurrence*[1] *of Fusarium conglutinans in the young roots and hypocotyl of susceptible Hollander, Wisconsin Hollander, and Wisconsin Ballhead cabbage seedlings grown on agar plates at 24° C.*

Period of exposure (days)	Susceptible Hollander						Wisconsin Hollander						Wisconsin Ballhead					
	Plant no.	Root			Hypocotyl		Plant no.	Root			Hypocotyl		Plant no.	Root			Hypocotyl	
		Root tip	Cortex	Stele	Cortex	Stele		Root tip	Cortex	Stele	Cortex	Stele		Root tip	Cortex	Stele	Cortex	Stele
2	1	0	+	0	0	0	1	0	0	0	0	0	1	0	+	0	+	0
	2	0	+	0	+	0	2	0	0	0	+	0	2	+	+	0	0	0
	3	0	+	0	+	0	3	0	+	0	0	0	3	0	+	0	0	0
	4		+	0	+	0	4	0	0	0	+	0	4	0		0		0
	5	+	+	0	+	0	5	+	0	0	+	0	5	0	0	0	0	0
	6		+	0	0	0	6	0	0	0	0	0						
	7		+	0	+	0	7	0	+	0	0	0						
	8	0	0	0	0	0	8	0	0	0	+	0						
	9	0	0	0	0	0	9	0	0	0								
	10	0	0	0	0	0	10	0	0	0	0	0						
	11	+	0	0	+	0												
	12	+	++	0		0												
	13		+	0	0	0												
3	14	0	++	0	+	0	11	+	+	0	0	0	6	++	+	++	0	0
	15	0	++	+	++	0	12	0	0	0	++	+	7	0	+	0	+	0
	16	++	+	0	0	0	13	0	0	0	0	0	8	0	+	0	0	0
	17		++	0	0	0	14	0	+	0	0	0	9	0	+	0	++	0
	18	+	++	+	+++	+	15	++	+	0	0	0						
							16	+++	++	+	+	0						
							17	+++	+	+	+	0						
							18	+++	++	+	+	0						
							19	0	+	0	0	0						
							20	++	+	0	0	0						
							21	0	+++	++	++	0						
							22	0	+	0	0	0						
							23	+	+	0	0							
							24	++	++	0								
5	19	++	+++	+++	+++	+++	25		+	0	++	+	10	0	++	0		
	20	++	+++	+++	++	+++	26	0	+	0	++	0	11		+	0	++	+
	21	++	+++	++	+++	+++	27	+	0	0			12	0	+	0	+	0
	22	+++	+++	+++	+++	+++	28	+++	++	+	+	0	13	0	+	0	++	0
	23		++	+	+++	+++	29		+++	++	++	0						
	24	+++	++	++	+++	+++	30	0	0	0	++	0						
	25	+++	+++	+++	+++	+++	31	0	+	0	++	0						

[1] The symbols used in this table and in tables 2 and 3 denote the degree of infection as follows: 0=no infection; +=slight infection; ++=moderate infection; +++=severe infection.

In table 2 the results in unsterilized inoculated soil are given. After 6 days the susceptible plants were generally infected in cortex and stele. Most Wisconsin Hollander plants were infected in the root cortex and over half of them showed the fungus in the stele. In Wisconsin Ballhead, infection was general in the cortex but only an occasional plant showed stelar infection. Essentially similar results were secured when sterilized inoculated soil was used.

TABLE 2.—*Occurrence*[1] *of Fusarium conglutinans in the young roots of susceptible Hollander, Wisconsin Hollander, and Wisconsin Ballhead cabbage plants grown from germinating seeds sown directly in unsterilized inoculated soil held at 22°–24° C. for various intervals*

Interval (days)	Susceptible Hollander			Wisconsin Hollander			Wisconsin Ballhead		
	Plant no.	Degree of infection in—		Plant no.	Degree of infection in—		Plant no.	Degree of infection in—	
		Cortex	Stele		Cortex	Stele		Cortex	Stele
3	1	0	0	1	0	0	1	0	0
	2	+	0	2	0	0	2	0	0
	3	+	0	3	0	0	3	0	0
	4	+	+	4	+	0	4	+	0
6	5	++	+	5	++	+	5	++	0
	6	++	+	6	++	0	6	++	+
	7	+	+++	7	++	0	7	++	0
	8	++	+	8	0	0	8	+	0
10				9	++	++	9	0	0
				10	++	+			
				11	+	+			
				12	+	0			
	9	++	+	13	+	0	10	+	0
	10		+++	14		0	11	++	+
14				15	++	+++	12	+	0
				16	++	++	13	++	0
				17	++	++	14	+	0
				18	+	0	15	+	0
				19		++			
				20	+	0	16	+	0
18				21	+	0	17	+	0
				22	++	+	18	0	0
							19	+	0

[1] For explanation of symbols see footnote to table 1.

In the examination of plants removed from the soil at various intervals there was a definite relationship between secondary root development and resistance. No secondary roots developed in the susceptible strain at 6, 10, and 14 days, and after the last period all were dead. The primary roots were brownish in color and had a collapsed appearance. It is to be seen in table 2 that at 6 days infection of this strain had occurred and the fungus had advanced into the stele. Undoubtedly it attacked the secondary roots before they emerged. In Wisconsin Hollander (intermediate resistant) plants at 6 days the primary roots were partially discolored and secondary roots were beginning to emerge. Root injury was more apparent as time went on, until at 18 days the only plants remaining turgid were those supported by secondary roots. At 6 days the primary roots of Wisconsin Ballhead (homozygous resistant) plants were normal in color and possessed well-developed secondary roots. Some injury to the primary root was apparent at 14 days and later but the secondary roots were normal in color and in turgidity.

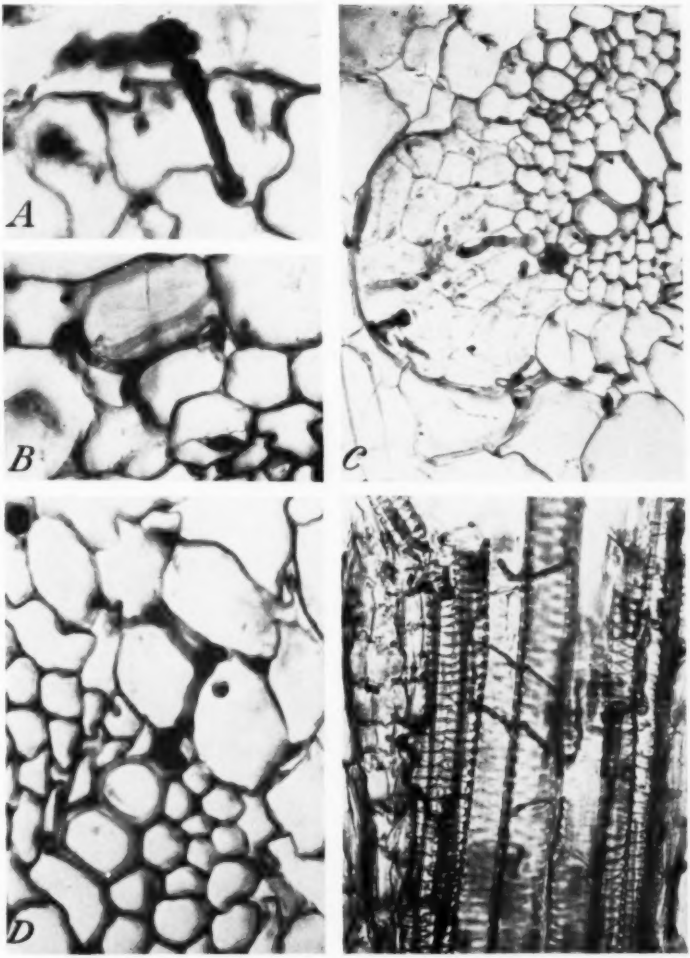
In table 3 are the results from 30-day-old seedlings transplanted to inoculated soil and grown at a soil temperature of 22° C. for various intervals. In these plants the roots were sectioned near the soil level where secondary roots were abundant. All plants of susceptible Hollander not previously sectioned were dead at the end of 17 days. Infrequent slight infection occurred in Wisconsin Hollander plants up to 14 days. At 17 days and longer moderate infection was common wherein a few vessels located in one section of the root were invaded. This type of localized infection of the xylem is in accord with the common field symptoms of this variety. In such cases unilateral disease development occurs or one or more lower leaves are affected and later drop off, leaving the plant without further outward sign of the disease (*1*). It is this type of infection which gives to Wisconsin Hollander the appearance of intermediate resistance or tolerance to the parasite. At high soil temperatures (24° C. or above) this resistance in Wisconsin Hollander is broken down and the fungus is no longer limited in its development. In this series infection was not found in the roots of homozygous resistant Wisconsin Ballhead.

TABLE 3.—*Occurrence* [1] *of Fusarium conglutinans in susceptible Hollander, Wisconsin Hollander, and Wisconsin Ballhead cabbage when 30-day-old seedlings were transplanted into infested soil and held at 22° C. soil temperature for various intervals*

Interval (days)	Susceptible Hollander		Wisconsin Hollander		Wisconsin Ballhead	
	Plant no.	Degree of infection	Plant no.	Degree of infection	Plant no.	Degree of infection
7	1	+	1	0	1	0
	2	+++	2	0	2	0
	3	+++	3	+		
9	4	++	4	0		
	5	+++	5	0		
	6	+++	6	0		
11	7	++	7	0	3	0
	8	+++	8	0	4	0
	9	+++	9	0	5	0
			10	+		
14	10	+++	11	0	6	0
	11	+++	12	0	7	0
	12	+++	13	0	8	0
17			14	0	9	0
			15	+	10	0
			16	++	11	0
20			17	++	12	0
			18	++	13	0
24			19	0	14	0
			20	++	15	0

[1] For explanation of symbols see footnote to table 1.

It is evident from this comparative study that prompt infection of cortex and stele of susceptible Hollander plants occurs. The infection of Wisconsin Ballhead roots is usually found to be limited to the epidermis and cortex of roots and hypocotyl. Seldom does the fungus increase in the cortex to the same degree that it does in susceptible plants. Such invasions apparently cause little damage and disappear with the sloughing of the cortex. Occasionally the fungus was found to have reached the stele in this variety but, with one exception, infection in the stele was very slight. In the one exceptional case the root tip was severely attacked and the fungus advanced a short distance into the stele. Cortical infection was generally more



SUSCEPTIBLE HOLLANDER ROOTS.

A, Penetration of the root epidermis in which there is an enlargement of the fungus hypha at the surface and invasion of the cell wall by a peglike constriction, × 1015; *B*, intracellular passage of cortical layer surrounding endodermis, the suberized thickening being shown in the wall of the infected cell, × 571; *C*, 10-day-old root showing infection of a young secondary root by hyphae invading from the surrounding cortex, × 365; *D*, 10-day-old root, showing a hypha crossing a cell of the pericycle and penetrating a protoxylem vessel, × 1015; *E*, longitudinal section through the stele of the root of a 10-day-old plant showing passage of the fungus through the thin-walled parts of spiral vessels and through pits of reticulate vessels, × 412.

severe in Wisconsin Hollander than in Wisconsin Ballhead. Infection of the stele was more frequent and the fungus advanced higher in the root. Thus this strain showed an intermediate degree of susceptibility or resistance to invasion which corresponds to the intermediate resistance commonly observed in the field.

PATHOLOGICAL ANATOMY OF THE CABBAGE ROOT

PENETRATION

Smith and Walker (*9*) studied penetration in plants grown on artificial media. They reported penetration chiefly in the embryonic region of the root, at times through root injuries, and infrequently by way of root hairs. The method of entrance was found to be intercellular and infrequently intracellular. In the present study no essential difference in penetration or subsequent development of the fungus was noticed between plants grown on soil-extract agar and those grown in inoculated soil. Penetration occurred within a period of 2 days in susceptible plants grown on agar at 24° C. and within 3 days in plants grown in inoculated soil at 22° (table 1). In the soil cultures infection occurred earlier and in a greater percentage of susceptible plants than in either of the resistant strains (tables 2 and 3). No difference in the process of penetration was noticed in the three strains of cabbage.

Infection of root hairs (fig. 1, *A*) is seldom found and is probably of no importance as an avenue of entrance to the host. Penetration occurs often in the root tip of susceptible plants, especially by way of the rootcap. The fungus often masses between and in the loose cells of the rootcap and continues upward to infect the growing tip. Often the growing point is not attacked and the infected root cap cells remain along the sides of the young root as the tip continues its growth.

Penetration is not limited to the young embryonic root structures but also occurs in the epidermis and outer cortical cells of the young root and hypocotyl. Through this avenue of entrance infection often occurs while the root tips remain free from infection (table 1). Infrequently the young root escapes the fungus and infection occurs only in the hypocotyl region. Sometimes infection through the hypocotyl in 30-day-old transplants was noticed in susceptible Hollander. The fungus also may gain entrance where secondary roots break through the cortex (pl. 2, *C*).

Direct intercellular and intracellular penetration (fig. 1, *A* to *D*; fig. 2, *B*, *D*) occur equally often, both in the young root and in the hypocotyl, while in the rootcap penetration is generally intercellular. In penetrating the epidermis the fungus generally neither shows change in thickness nor becomes distorted as if exerting pressure. Occasionally a cell wall is distended (fig. 1, *B*) as if from pressure. Infrequently a strand becomes thickened at the cell surface and penetrates by a smaller projection through the cell wall (pl. 2, *A*).

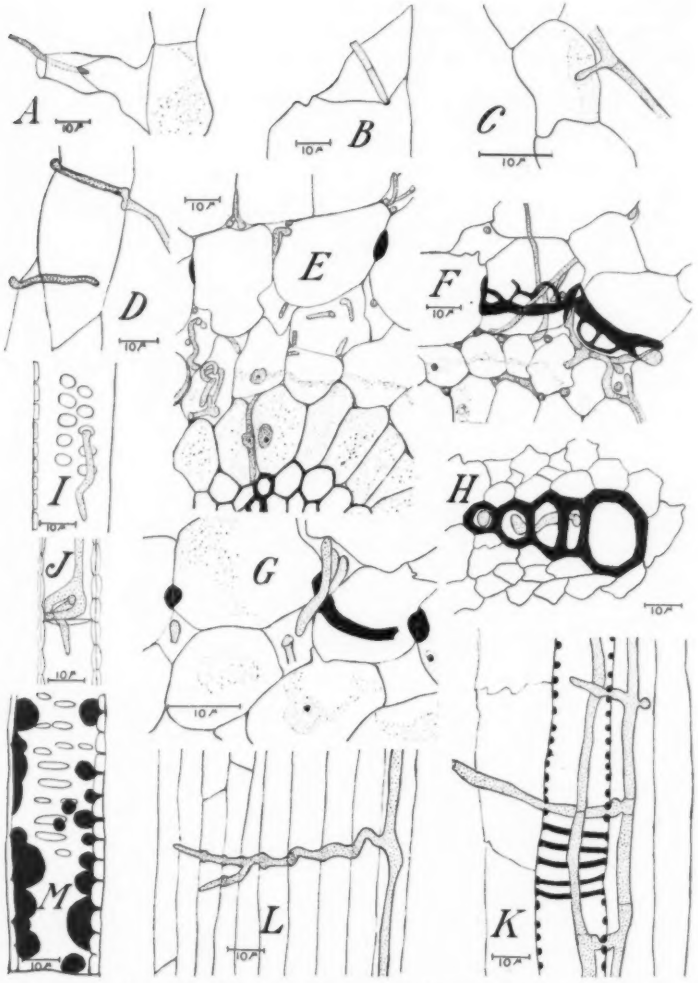


FIGURE 1.—Penetration and development of *Fusarium conglutinans* in cabbage roots: *A*, Fungus entering through root hair; *B*, penetration in which the inner cell wall is distended, an infrequent occurrence; *C*, *D*, direct penetration through epidermis; *E*, fungus in inner cortex and pericycle; *F*, cortical cells with suberized thickenings next to the endodermis being traversed intracellularly; *G*, an infrequent occurrence where the fungus forces passage between two cells of the layer surrounding the endodermis (note that the suberized thickenings do not meet in these two cells); *H*, fungus passing laterally through a vessel by way of border pits; *H*–*K*, fungus in vessels, entrance having been effected through the thin border pits by a conforming constriction of the hyphae; *L*, fungus passing laterally through several cells of the phloem, with bending of the hypha and enlargement near the cell wall as if due to stress and resistance in penetrating walls of this tissue; *M*, orientation and formation of occlusions in a vessel. (All drawings are from susceptible Hollander plants with the exception of *D*, which is from Wisconsin Hollander.)

SUBSEQUENT COURSE OF INVASION

INVASION THROUGH THE CORTEX

In the embryonic roots of susceptible plants, as reported by Smith and Walker (*9*), the fungus readily makes its way to the vascular system. The embryonic growing point is easily attacked and the root is sometimes stunted, especially when infection by the fungus occurs en masse through the rootcap. More often, however, infection takes place through the epidermis and cortex of the root (table 1).

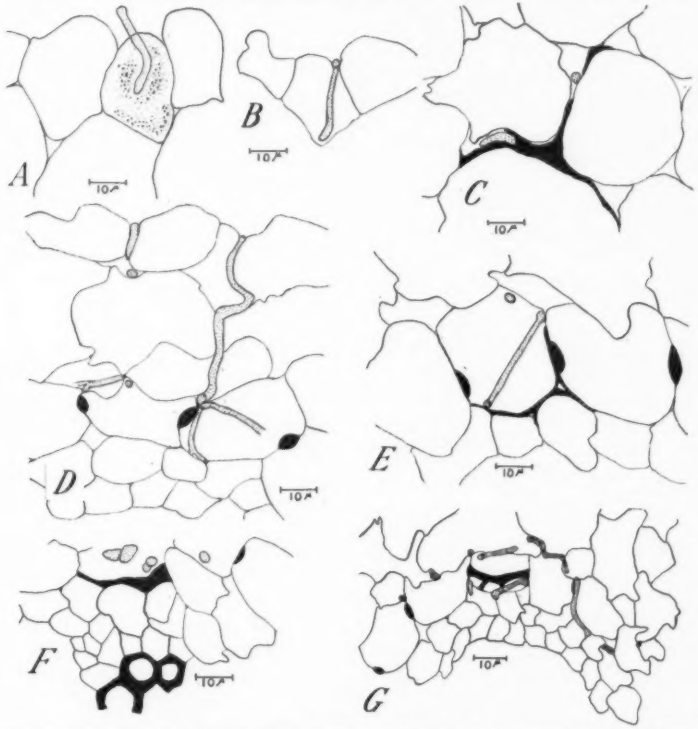


FIGURE 2.—Penetration and development of *Fusarium conglutinans* in homozygous resistant Wisconsin Ballhead cabbage: *A*, Penetration of epidermal cell accompanied by granulation of the cell contents; *B*, direct penetration of epidermal cell; *C*, deposition of gumlike material between cortical cells in advance of the fungus, an infrequent reaction not effective in preventing deeper invasion into the cortex; *D*, Fungal strand extending through cortex to endodermis, penetration being intercellular but the layer of cells surrounding the endodermis is crossed intracellularly; *E*, suberization of walls between endodermis and the surrounding layer of cortical cells; *F*, suberization between endodermis and pericycle, effectively barring the fungus from the stele; *G*, fungus in the cortex of a resistant root, the layer surrounding the endodermis being traversed intracellularly where radial suberized thickenings naturally occur, intercellular passage being likely to occur where these thickenings are lacking.

When epidermal calls are penetrated directly, the fungus soon becomes intercellular. It generally remains intercellular in its course across the cortex, although it sometimes occurs within and passes through cortical cells (fig. 1, *E;* pl. 1, *C*). The rigid suberized inner bands in the cells of the cortical layer surrounding the endodermis, with but few exceptions, inhibit intercellular passage. Intracellular penetration of this layer takes place readily, however, in old and young roots and in both susceptible and resistant plants when infec-

tion of the latter occurs (fig. 1, *F;* fig. 2, *D;* pl. 2, *B*). In one case (fig. 1, *G*) the fungus forced an intercellular passage, but in that case the thickenings on the adjacent cell walls did not make a good parallel fit. As stated above, these thickenings may be lacking or partly lacking, and in such cases intercellular penetration occurs (fig. 2, *G*). Thus, although it possesses suberized thickenings, this layer was not a barrier to radial ingress of the fungus in the cabbage varieties studied, as found by Smith and Walker (*9*) in other varieties.

Although *Fusarium conglutinans* is primarily a vascular parasite, it is often found in the cortex for a considerable distance in the root and hypocotyl, as a result of repeated infection and of vertical advance of the fungus. The large intercellular spaces make convenient runways for the hyphae. When repeated infection does not occur, the fungus advances highest in the cortex in the intercellular spaces just outside the endodermal layer. Sometimes the fungus advances higher in the cortex than in the stele. This was especially noticeable in the two resistant strains, and may be due to incompatibility of fungus and host in the vessels.

The fungus in the cortex is an important source of secondary infection. Secondary roots are frequently attacked before they completely traverse the cortex of the primary root (pl. 2, *C*). In several cases initial infection of the stele of the main root resulted from such invasion of secondary roots. The cortex of the primary root infrequently appears predisposed to initial penetration as the result of outward growth of secondary roots since the fungus sometimes invades this region as the secondary root is about to push through. It occasionally enters the cortex of the primary root after the latter has been ruptured by the secondary root.

Development in the Stele

The fungus apparently encounters more difficulty in passing the endodermis and pericycle than the cortical layer surrounding the endodermis. These tissues are usually traversed intracellularly. Generally the protoxylem vessels are next attacked, the fungus passing through the thin vessel wall between spiral thickenings. Plate 2, *D*, shows the passage of a strand through the pericycle into the protoxylem vessel. The vessels centripetally become reticulate and pitted and the fungus passes from one vessel to another through pits (fig. 1, *H–K;* pl. 2, *E*). The fungus seems to have an affinity for the protoxylem vessels, from which it repeatedly passes into the larger reticulate vessels.

The phloem and parenchymatous cells of the stele are also invaded by the fungus. In the phloem the fungal strands often appear much larger in cross section than in the xylem, apparently the result of the turning and enlargement of the strands in the process of penetration of cell walls. The crooked course of fungal strands with enlargement before wall penetration suggests that the fungus encounters more mechanical resistance in the phloem tissue. This phenomenon is noticed in both susceptible and resistant roots. Figure 1, *L*, shows a strand passing through several cells of the phloem.

Reaction of Host to Parasite

Although no structural differences in the three strains of cabbage were noticed, there were differences in host reaction in the presence of the fungus.

Protoplasmic Reaction

Frequently, when penetrated directly, the protoplasmic content of the cells became granular and stained deeply with Delafield's haematoxylin. The cells appeared to be killed, and often ruptured. Under such conditions the fungus sometimes became granular and showed various stages of disintegration. Often, however, it passed through such cells into the intercellular spaces, where it continued to grow with no subsequent deleterious reaction of host cells or parasite.

This host reaction, while not extremely common, was more frequent and more pronounced in plants growing on artificial media. It was more often observed in both resistant varieties than in the susceptible strain. Figure 2, *A*, shows penetration of an epidermal cell in a Wisconsin Ballhead plant followed by protoplasmic granulation. Plate 1, *D*, shows a similar reaction in Wisconsin Hollander.

This reaction is suggestive of that found in cereals resistant to rust, although in the latter case cells and fungus succumb as a result of incompatibility. The reaction in cabbage, although not of great importance since it neither occurs uniformly nor is effective in checking the fungus, is interesting in that it is more pronounced in resistant than in susceptible plants.

Cell-Wall Reaction

Occasionally the walls of cortical cells of Wisconsin Ballhead appeared to become suberized slightly in advance of the fungus, as indicated by their staining red with safranine (fig. 2, *C* and *E*). A similar reaction was more common in the endodermis and pericycle of both resistant strains and less so in susceptible plants. Figure 2, *F*, shows the wall between endodermis and pericycle of a Wisconsin Ballhead root heavily suberized. In plate 1, *E*, the inward advance of the fungus was checked by endodermal and pericyclic suberization in a Wisconsin Hollander plant. This localized suberization in plants of the two resistant strains was especially noticeable where the infected cortex was in the process of detachment, and appeared to be effective in limiting the ingress of the fungus into the stele.

Too much emphasis should not be placed on these forms of host reaction, since they do not occur uniformly in all plants. At times, however, they do apparently play an important role in excluding the fungus from the stele. The real seat of resistance must be found in the nature of the cell contents which are apparently not favorable to a vigorous invasion and development by the fungus in intermediate resistant and in homozygous resistant plants.

Occlusions

Deposits of gumlike material in xylem vessels and parenchyma of cabbage were discussed by Smith and Walker (*9*). Occlusions were noticed by them in both susceptible and resistant roots growing in soil, but only in resistant roots growing on artificial media. In the present study infrequent occlusions were found in all strains, in both soil cultures and plants grown on artificial media. Occluded vessels were found most often in the series in which germinating seedlings were placed directly in inoculated soil. These seedlings after 10 to 18 days exhibited various degrees of injury in which cortex and even parts of the stele were lacking in young roots. Throughout the study occlu-

sions were seldom observed in the same vessels with fungal strands but were more often found in adjacent vessels. They also occurred where no trace of the fungus could be found. Their formation is shown in figure 1, *M*. Droplets of a gumlike substance apparently have their beginning at the pits of the vessel and may increase until they completely plug the latter. Generally but few vessels of an infected root show these gumlike deposits.

DISCUSSION

In this investigation a study of the intimate relation of the yellows organism to susceptible and resistant hosts has been undertaken. Stable resistance, controlled by a single dominant gene (Wisconsin Ballhead), was compared with a definite but more variable type not completely dominant and controlled apparently by a number of factors which merely modify the expression of the gene for susceptibility (Wisconsin Hollander).

In an earlier paper Smith and Walker (*9*) reported a similar study of susceptible lines and homozygous resistant lines of different origin. They were unable to determine any morphological basis for resistance. Tisdale (*11*), working with flax wilt (*Fusarium lini* Bolley), noted in the resistant strains a tendency of the cell walls of the cortex to thicken and show signs of suberization in advance of the fungus. He regarded this as a partial explanation of resistance but believed that the interaction between hyphae and the host protoplasm was perhaps more important in checking the parasite. Fahmy (*3*) in a study of cotton wilt (*F. vasinfectum* Atk.) described penetration through the root cap, while Dharmarajulu (*2*) considered resistance to be based upon a combination of cell-wall reaction and antagonism of the protoplast.

The writers have found that penetration in susceptible Hollander cabbage and in the two resistant strains proceeds in essentially the same manner. In all of these strains there appears to be more penetration of the cortex of root and hypocotyl than was found in other strains of cabbage by Smith and Walker (*9*). Moreover the cell-wall thickenings of the inner cortical layer of the root do not obstruct fungus ingress into the stele since the parasite penetrates these cells intracellularly.

While under the conditions of these studies there was invasion of the cortex of a larger percentage of resistant roots than had been reported for the lines studied earlier (*9*), invasion of the stele in the homozygous resistant Wisconsin Ballhead was rare and meager in extent. The parasite was obviously retarded in the cortex. This inhibition of growth was not correlated with any consistent morphological or biochemical reaction which could be demonstrated by histological methods although cell-wall suberization and granulation of the protoplasm occasionally occurred. These reactions, it is true, were somewhat more frequent in the resistant lines than in the susceptible line, but they were nevertheless too infrequent to account for the marked and distinct resistance which occurs in Wisconsin Ballhead.

At the outset of this study it was thought that since the resistance in Wisconsin Hollander had been shown to be distinct in its genetical basis from that of Wisconsin Ballhead, it might show a distinct histological picture. The results presented herein, however, indicate that the intermediate type of resistance exhibited by this strain differs in

degree rather than in kind from that of the homozygous resistant line. The relation of the parasite to this host is of the same order. There are, again, no consistent morphological reactions which can be interpreted as the basic cause of resistance. The fungus is more active in the cortex and is not so consistently excluded from the stele as is the case with Wisconsin Ballhead. Whether or not the same basic factors, in a lesser degree, as those which occur in Wisconsin Ballhead are responsible has not been demonstrated. It is clear, however, that whatever the nature of resistance may be in either resistant strain, it is not readily discerned by histological methods. It may be due to antagonistic compounds in the host cell or it may be simply an inherent inability on the part of the parasite to live and thrive in the host cell as successfully as it does in that of the susceptible strains. It would appear that further knowledge of the nature of resistance to *Fusarium conglutinans* can be secured best through a biochemical approach to the question.

SUMMARY

A comparative study was made of the invasion of three strains of Hollander cabbage by the yellows organism (*Fusarium conglutinans* Wr.): (1) a very susceptible strain of Hollander, (2) an intermediate resistant type (Wisconsin Hollander), and (3) a homozygous resistant strain (Wisconsin Ballhead).

The fungus enters the three strains of cabbage through the root tip and through the cortex of the young root and hypocotyl. Penetration of cell walls is apparently accomplished by mechanical pressure.

The fungus traverses the cortex of susceptible roots readily and often persists in this tissue as high as the upper hypocotyl. Secondary roots are often attacked by the pathogen as they push through the cortical tissue.

Penetration of Wisconsin Ballhead was quite frequent but the fungus was generally limited to the outer cortical cells or the lower root-tip region and very seldom reached the vascular system.

In Wisconsin Hollander the pathogen was retarded but not to the same extent as in Wisconsin Ballhead. Infection of the vascular system occurred more often but here the fungus was usually restricted to a few localized vessels.

No definite morphological differences occur in the strains of cabbage studied; however some differences in reaction of the host to the fungus were apparent in the resistant strains.

Only in Wisconsin Ballhead was intercellular suberization of cortical cells in advance of the fungus noted. Suberization of endodermis and pericycle walls often occurred locally in both Wisconsin Ballhead and Wisconsin Hollander when these tissues were approached by the fungus. Granulation of epidermal and outer cortical cells was noticeable in certain plants of Wisconsin Ballhead and Wisconsin Hollander when these cells were penetrated directly.

The basis of resistance in Wisconsin Hollander appears to differ in degree rather than in kind from that of Wisconsin Ballhead. Whatever the true nature of resistance may be, it is apparently not readily discernible by histological methods, and further knowledge regarding it may best be sought through a biochemical approach to the question.

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NUTRIENT VALUE OF THE PHOSPHORUS IN CALCINED PHOSPHATE AS DETERMINED BY GROWTH OF PLANTS IN GREENHOUSE EXPERIMENTS [1]

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INTRODUCTION

The complex minerals fluorapatite ($Ca_{10}F_2(PO_4)^6$) and hydroxyfluorapatite ($Ca_{10}(GH, F)(PO_4)^6$) are the principal phosphatic constituents of nearly all commercial varieties of phosphate rock mined throughout the world (*11, 13*).[2]

All commercial varieties and grades of domestic phosphate rock contain about 3 to 4 percent of fluorine (*13*), and the percentage of fluorine in the rock is invariably higher than that corresponding to the fluorapatite equivalent of the total phosphorus content. The state of combination of the excess fluorine is not definitely known, but the available evidence indicates that it is probably present chiefly as calcium fluoride. If it is assumed that all the phosphorus in domestic phosphate rock is present in the form of fluorapatite, the fluorine in excess of that corresponding to the fluorapatite equivalent of the total phosphorus amounts to approximately 10 to 30 percent of the total fluorine. Therefore, about 70 to 90 percent of the fluorine is equally distributed between the two atoms of fluorine in the fluorapatite; for convenience these may be designated as the first (F_B) and second (F'_B) atoms of fluorine. Finally, the fluorine in excess of that corresponding to the second (F'_B) atom of fluorine in the fluorapatite equivalent of the phosphorus amounts to about 55 to 65 percent of the total fluorine.

Recent laboratory studies (*17, 22, 23*) have shown that when domestic phosphate rock, containing about 5 to 10 percent of silica, is heated in the presence of water vapor for 30 minutes at 1,375° to 1,425° C. it is possible to volatilize upward of 95 percent of the total fluorine (corresponding to upward of 86 percent of the F'_B fluorine) and convert 80 percent or more of the phosphorus into the citrate-soluble (available) condition. In order to obtain efficient reaction in the minimum time, the process must be regulated to give the maximum degree of contact between the water vapor and the particles of phosphate rock.

In experiments (*22*) with Florida land-pebble phosphate, containing 3.67 to 3.85 percent of fluorine and 6.80 to 10.85 percent of silica,

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[2] Reference is made by number (italic) to Literature Cited, p. 847.

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volatilization of less than about 65 percent of the total fluorine (corresponding approximately to the fluorine in excess of the F'_n fluorine) was accompanied by a decrease in the citrate solubility of the phosphorus in the product, as compared with that of the phosphorus in the original rock. From that point, however, the citrate solubility of the phosphorus corresponded approximately to the percentage of the F'_n fluorine volatilized, provided the temperature of the experiment exceeded 1,300° C. The defluorinated material (calcined phosphate) contains little or no water-soluble phosphorus. The chemical nature of the citrate-soluble phosphorus has not been definitely determined, but it is believed to consist principally of the high-temperature form of tricalcium phosphate (*3*, *26*).

Various processes have been proposed for the manufacture of available phosphates by heating phosphate rock with alkali salts, usually the sulphates or carbonates of sodium and potassium. These processes have been discussed by Fishburne (*6*), Guernsey and Yee (*8*), and Waggaman and Easterwood (*27*). As yet, attempts to operate such processes on a commercial scale have not met with sustained success in this country. In Germany, however, a product known as Rhenania phosphate (*18*, *24*) has been manufactured for some years by heating phosphate rock with alkali salts under certain conditions. In this country Allison, Braham, and McMurtrey (*1*), Conner (*4*), Haskins (*9*, *10*), and Mooers (*19*), and, in Europe, Erdély (*5*), Gerlach and Nolte (*7*), Jakobsen (*16*), Niklas, Strobel, and Scharrer (*20*), and Ràth (*21*) have shown that citrate-soluble phosphates, prepared by heating phosphate rock with alkali salts, are excellent sources of phosphorus for plant growth.

Inasmuch as the process which involves the heating of silica-bearing phosphate rock in the presence of water vapor (without the addition of alkali salts) seems to have commercial possibilities, greenhouse pot experiments were carried out on four types of soil in order to obtain information on the nutrient value of calcined phosphates in which the phosphorus showed a wide range of citrate solubilities. The Alabama, Arkansas, and West Virginia Agricultural Experiment Stations and the Bureau of Plant Industry, United States Department of Agriculture, cooperated in the greenhouse experiments. The results are presented in this paper.

MATERIALS AND EXPERIMENTAL METHODS

Data on the preparation and composition of the phosphates are given in table 1.

Samples 3 and 4 were commercial phosphate rock from a deposit of unknown location in the Florida land-pebble phosphate district. The colloidal phosphate (sample 5) was a natural claylike material (*12*, *13*) from an abandoned phosphate-washer waste pond in the Florida hard-rock phosphate district. The Tennessee brown-rock phosphate (sample 6) was a commercial kiln-dried material from a deposit near Mountpleasant, Tenn.

Samples 7, 8, 9, and 10 (calcined phosphates) were prepared in a laboratory tube furnace by heating Florida land-pebble phosphate rock, of the general composition represented by samples 3 and 4, in the presence of water vapor.[3] In this rock, the fluorine in excess

[3] These samples were prepared by D. S. Reynolds, Fertilizer Investigations, Bureau of Chemistry and Soils.

of that corresponding to the second (F'_B) atom of fluorine in the fluorapatite equivalent of the total phosphorus, amounted to approximately 63 percent of the total fluorine. In confirmation of previous results (*22*), no increase in the citrate solubility of the phosphorus was obtained until all the fluorine in excess of that corresponding to the second (F'_B) atom of fluorine in the fluorapatite equivalent of the total phosphorus was volatilized. Volatilization of only 50 percent of the total fluorine was accompanied by a decrease in the citrate solubility of the phosphorus in the product (sample 7), as compared with that of the phosphorus in the original rock.

TABLE 1.—*Phosphate materials used in greenhouse experiments*

Sample no.	Phosphate material	P_2O_5		Availability of P_2O_5	Total fluorine content	Total fluorine volatilized	F'_B fluorine [2] volatilized
		Total	Available [1]				
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Florida pebble superphosphate, 20-mesh	20.40	[3] 20.32	99.6	1.65	[4] 31	
2	Dicalcium phosphate, $CaHPO_4 \cdot 2H_2O$, c. p. material, 80-mesh	41.52	41.52	100.0	0.00		
3	Florida pebble phosphate, untreated material, 200-mesh	31.25	4.12	13.2	3.85		
4	Florida pebble phosphate, same as no. 3 except 100-mesh	31.39	3.64	11.6	3.85		
5	Colloidal phosphate, 100-mesh	23.03	2.39	10.4	1.92		
6	Tennessee brown-rock phosphate, untreated material, 100-mesh	33.77	2.58	7.6	3.67		
[5] 7	Calcined phosphate; 10-g charge of 200-mesh Florida pebble heated 0.5 hour at 1,300° C	33.88	2.30	6.8	2.08	49.9	0.0
[5] 8	Calcined phosphate; 10-g charge of 200-mesh Florida pebble heated 0.5 hour at 1,400° C	34.09	7.01	20.6	1.49	64.2	1.6
[5] 9	Calcined phosphate; 10-g charge of 40- to 80-mesh Florida pebble heated 1.0 hour at 1,400° C	33.38	16.39	49.1	.85	78.9	44.0
[5] 10	Calcined phosphate; 5-g charge of 40- to 80-mesh Florida pebble heated 1.0 hour at 1,400° C	33.78	26.39	78.1	.27	93.3	82.2
[6] 11	Calcined phosphate, prepared from Tennessee brown-rock phosphate	33.97	22.21	65.4	.40	[4] 90.0	[4] 76.0
[6] 12	do	34.21	29.46	86.1	.10	[4] 97.0	[4] 93.0
[6] 13	do	35.09	32.52	92.7	.10	[4] 97.0	[4] 93.0

[1] Neutral ammonium citrate method.
[2] Fluorine corresponding to the second atom of fluorine in the fluorapatite equivalent of the total phosphorus.
[3] Contained 17.5 percent of water-soluble P_2O_5.
[4] Approximate figure.
[5] Ground to 100-mesh.
[6] Ground to 80-mesh.

Samples 11, 12, and 13 (calcined phosphates) were prepared in an experimental direct-fired rotary kiln from a commercial grade of Tennessee brown-rock phosphate.[4]

The conditions of the greenhouse experiments are outlined in table 2. In all experiments the potash and phosphate fertilizers were uniformly mixed with the entire mass of soil just before the seed was sown or the plants (cabbage) were transplanted; the initial applications of nitrogen fertilizers were made in the same manner. For a given series of experiments, the phosphates were applied on the basis of equal quantities of total phosphoric oxide (P_2O_5). In the second series of experiments by the Department of Agriculture chemically pure calcium carbonate, at the rate of 2,000 pounds per acre, was applied with the nitrogen, phosphate, and potash fertilizers to one set of pots; in contrast to the first and third series, the plants in this series were harvested before the seed heads were formed.

[4] These samples were supplied by Paul Caldwell, Moorman Manufacturing Co., Quincy, Ill.

TABLE 2.—*Conditions of greenhouse experiments*

Item	Alabama Experiment Station	Arkansas Experiment Station	U. S. Department of Agriculture	West Virginia Experiment Station	
Replications.....number	2	3	3	2	3.
Soil type and pH	Cecil clay, pH 6.0.	Clarksville silt loam, pH 6.23.	Norfolk loamy fine sand, pH 4.8.	Dekalb silt loam, pH 5.25.	Dekalb silt loam, pH 6.10.
Weight of air-dry soil per pot.....kilograms	8 [1]	10 [1]	5 [2]	7 [1]	7.[1]
P_2O_5 application [3].....grams per pot	0.64	0.334	0.6	0.35	0.35.
P_2O_5 application [3].....pounds per acre	160	66.8	240	100	100.
Nitrogen fertilizer.....do	$NaNO_3$, 600 [4]	$(NH_4)_2SO_4$, 300	$NaNO_3$, 260; $(NH_4)_2SO_4$, 195.	Urea, 300 [5]	Urea, 200; $NaNO_3$, 500.[6]
Potash fertilizer.....do	KCl, 100.	KCl, 300.	Manure salts (27.5 percent K_2O), 436.	KCl, 100.	KCl, 100.
Crop	Cabbage	Sudan grass	Millet	Millet	Sudan grass.
Date of seeding	Feb. 28, 1934 [7]	June 21, 1934	Jan. 25, 1934 [8]	May 7, 1934	Aug. 15, 1934.
Plants per pot.....number	4	12	10	6	6.
Date of harvesting	Apr. 24, 1934	Aug. 8, 1934	Apr. 3, 1934 [9]	Aug. 7, 1934	Oct. 31, 1934.

[1] 2-gallon pots.
[2] 1-gallon pots.
[3] Total P_2O_5.
[4] 300 pounds at transplanting and 300 pounds as top dressing.
[5] 200 pounds at seeding and 100 pounds as top dressing when plants were about half grown.
[6] Applied as top dressing on Oct. 2, 1934.
[7] Date of transplanting.
[8] First series; second and third series planted July 21 and Aug. 1, 1934, respectively.
[9] First series; second and third series harvested Aug. 28 and Sept. 24, 1934, respectively.

EFFECT OF PHOSPHATES ON OVEN-DRY WEIGHT AND PHOSPHORUS CONTENT OF PLANTS

The average oven-dry weight and phosphorus content of the plants are given in table 3.[5] All of the soils were deficient in available phosphorus, and marked increases in the growth and phosphorus content of the plants were invariably obtained by the application of either superphosphate, dicalcium phosphate, or certain of the calcined phosphates. For a given series of experiments, the effect of the phosphates on the phosphorus content of the plants was, with a few exceptions, in the same order as their effect on the dry weight of the plants.

At the Alabama station, the 200-mesh Florida pebble phosphate rock was an excellent source of phosphorus for cabbage on Cecil clay of pH 6.0. At the West Virginia station, this material was also an excellent source of phosphorus for millet on Dekalb silt loam of pH 5.25, whereas it gave very poor results with Sudan grass on Dekalb silt loam of pH 6.10. Furthermore, the ground phosphate rock was not an efficient source of phosphorus for millet on Norfolk loamy fine sand of pH 4.8 (U. S. Department of Agriculture) and for Sudan grass on Clarksville silt loam of pH 6.23 (Arkansas station). Under comparable conditions, the 200-mesh Florida pebble phosphate appeared to be somewhat more efficient than the 100-mesh material. The Florida pebble phosphate gave better results than did either the colloidal phosphate or the Tennessee brown-rock phosphate. Under the conditions of these experiments, the nutrient value of the ground phosphate rock was not correlated with either the type or pH of the soil or the type of crop grown.

When the phosphate rock was calcined in such a manner as to volatilize only 50 percent of the flourine the product (sample 7) was invariably less effective than the untreated rock in promoting plant growth and absorption of phosphorus. Volatilization of larger percentages of the flourine usually had a marked progressive effect in increasing the nutrient value of the phosphorus (samples 8 to 13), the effects on plant growth and absorption of phosphorus being related, more or less directly, to the amount of flourine volatilized and to the corresponding citrate solubility of the phosphorus. In general, calcined phosphates in which the availability of the phosphorus amounted to 78 percent or more (samples 10, 12, and 13), gave as good or better results than did superphosphate.

Addition of calcium carbonate at the rate of 2,000 pounds per acre (second series, U. S. Department of Agriculture) did not have a pronounced effect on the weight of millet plants fertilized with either superphosphate, dicalcium phosphate, or calcined phosphate, but caused a marked reduction in the weight of those receiving either colloidal phosphate or untreated Tennessee brown-rock phosphate. Liming increased the total absorption of phosphorus by the plants from the no-phosphorus pots, but decreased the absorption by plants from the phosphate-treated pots.

The highest recovery of the applied phosphorus, as indicated by the percentage absorption of the phosphorus by the plants (table 3), was obtained in the experiments at the Arkansas station, followed in

[5] The writers are indebted to J. B. Martin, Soil Fertility Investigations, Bureau of Plant Industry, and L. F. Rader, Jr., Fertilizer Investigations, Bureau of Chemistry and Soils, for assistance in making the phosphorus determinations.

order by the experiments at the West Virginia and Alabama stations and the United States Department of Agriculture. Excluding the limed-soil series (U. S. Department of Agriculture), the maximum recovery of the applied phosphorus was 43.9 percent (sample 11, calcined phosphate, Arkansas station) and the minimum recovery was 0.08 percent (sample 7, calcined phosphate, first series, U. S. Department of Agriculture). In general, the percentage recovery of the applied phosphorus decreased with increase in the per acre rate of application of the phosphate.

TABLE 3.—*Average oven-dry weight and P_2O_5 content of plants and recovery of applied P_2O_5* [1]

OVEN-DRY WEIGHT

Sample no.	Phosphate treatment: Material	Millet: Cabbage, Alabama Experiment Station [2]	Millet: U. S. Department of Agriculture [3] — Second series: First series	Millet: U. S. Department of Agriculture [3] — Second series: Unlimed	Millet: U. S. Department of Agriculture [3] — Second series: Limed [4]	Millet: U. S. Department of Agriculture [3] — Third series	Millet: West Virginia Experiment Station [5]	Sudan grass: Arkansas Experiment Station [6]	Sudan grass: West Virginia Experiment Station [5]
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
	No phosphorus	0.95	3.05	1.85	2.30	5.60	17.8	10.8	5.7
1	Superphosphate	16.15	9.25	6.75	6.60	13.45	30.1	32.7	14.5
2	Dicalcium phosphate	12.80	10.30	7.90	8.10	16.70	31.1	31.9	16.5
3	Untreated Florida pebble phosphate, 200-mesh	14.75	6.20	.....	.....	11.40	30.7	23.7	7.5
4	Untreated Florida pebble phosphate, 100-mesh	.....	.....	.....	.....	10.40	.....	.....	7.1
5	Colloidal phosphate	.....	3.90	3.80	2.55	.....	.....	.....	.....
6	Untreated Tennessee brown-rock phosphate	.....	.....	3.35	2.00	7.55	.....	.....	6.9
7	Calcined phosphate, prepared from Florida pebble	3.50	3.70	.....	.....	6.50	23.0	14.7	7.4
8	do	11.00	6.05	.....	.....	10.50	26.3	20.1	9.3
9	do	14.65	10.50	.....	.....	12.15	27.6	24.3	12.8
10	do	17.50	11.50	.....	.....	14.75	30.4	28.3	15.6
11	Calcined phosphate, prepared from Tennessee brown rock	15.95	12.25	.....	.....	14.25	28.2	34.6	15.0
12	do	17.40	12.70	7.40	6.70	15.45	31.0	30.3	15.8
13	do	.....	.....	.....	.....	16.25	.....	.....	.....

AVERAGE P_2O_5 CONTENT [1]

		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
	No phosphorus	1.4	13.1	7.2	10.1	31.9	49.8	71.6	22.5
1	Superphosphate	42.4	48.9	32.4	25.7	70.6	91.8	169.4	58.8
2	Dicalcium phosphate	34.8	49.5	34.4	31.5	91.8	100.3	149.9	69.6
3	Untreated Florida pebble phosphate, 200-mesh	51.6	25.4	.....	.....	48.1	97.2	136.6	31.4
4	Untreated Florida pebble phosphate, 100-mesh	.....	.....	.....	.....	45.2	.....	.....	30.8
5	Colloidal phosphate	.....	18.6	14.8	11.2	.....	.....	.....	.....
6	Untreated Tennessee brown-rock phosphate	.....	.....	13.7	8.8	41.5	.....	.....	24.4
7	Calcined phosphate, prepared from Florida pebble	7.2	13.6	.....	.....	34.1	69.1	92.0	24.6
8	do	29.4	23.5	.....	.....	43.0	74.7	121.5	33.6
9	do	49.6	35.7	.....	.....	49.8	75.7	143.0	54.9
10	do	47.4	49.5	.....	.....	64.2	92.2	187.0	62.7
11	Calcined phosphate, prepared from Tennessee brown rock	61.6	45.3	.....	.....	55.6	84.1	218.1	62.6
12	do	63.5	67.4	37.3	30.4	67.2	106.6	176.9	67.8
13	do	.....	.....	.....	.....	73.9	.....	.....	.....

[1] Per pot.
[2] Total P_2O_5 applied at rate of 0.64 g per pot (160 pounds per acre).
[3] Total P_2O_5 applied at rate of 0.60 g per pot (240 pounds per acre).
[4] C. p. $CaCO_3$ applied at rate of 2,000 pounds per acre.
[5] Total P_2O_5 applied at rate of 0.35 g per pot (100 pounds per acre).
[6] Total P_2O_5 applied at rate of 0.334 g per pot (66.8 pounds per acre).

TABLE 3.—*Average oven-dry weight and P_2O_5 content of plants and recovery of applied P_2O_5*—Continued

AVERAGE RECOVERY OF APPLIED P_2O_5

Sample no.	Phosphate treatment: Material.	Cabbage, Alabama Experiment Station	Millet: U. S. Department of Agriculture: First series	Millet: U. S. Department of Agriculture: Second series: Unlimed	Millet: U. S. Department of Agriculture: Second series: Lime	Millet: U. S. Department of Agriculture: Third series	Millet: West Virginia Experiment Station	Sudan grass: Arkansas Experiment Station	Sudan grass: West Virginia Experiment Station
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Superphosphate	6.4	6.0	4.2	2.6	6.4	12.0	29.3	10.4
2	Dicalcium phosphate	5.2	6.1	4.5	3.6	10.0	14.4	23.4	13.5
3	Untreated Florida pebble phosphate, 200-mesh	7.8	2.1	.....	.....	2.7	13.5	19.5	2.5
4	Untreated Florida pebble phosphate, 100-mesh	.....	.....	.....	.....	2.2	.....	.....	2.4
5	Colloidal phosphate	.....	.9	1.3	.2	.....	.....	.....	.....
6	Untreated Tennessee brown-rock phosphate	.....	.....	1.1	−.2	1.6	.....	.....	.5
7	Calcined phosphate, prepared from Florida pebble	.9	.08	.....	.....	.4	5.5	6.1	.6
8	do	4.4	1.7	.....	.....	1.8	7.1	14.9	3.2
9	do	7.5	3.8	.....	.....	3.0	7.4	21.4	9.3
10	do	7.2	6.1	.....	.....	5.4	12.1	34.6	11.5
11	Calcined phosphate, prepared from Tennessee brown rock	9.4	5.4	.....	.....	3.9	9.8	43.9	11.5
12	do	9.7	9.1	5.0	3.4	5.9	16.2	31.5	12.9
13	do	.....	.....	.....	.....	7.0	.....	.....	.....

RELATIVE EFFICIENCIES OF THE PHOSPHATES

The relative efficiencies of the phosphates, as indicated by the increase in the dry weight and phosphorus content of the plants, are summarized in table 4. In this table the efficiencies of the phosphates are based upon 100 as the increase in dry weight or phosphorus content due to the application of superphosphate. Although the individual series of experiments showed some discrepancies between the relative efficiencies of the phosphates as determined by the increase in the dry weight and the phosphorus content, respectively, the average efficiencies (table 4), as determined in this way, were, for the most part, in very good agreement. In general, the average efficiency as determined by the increase in dry weight was somewhat higher than that determined by the increase in phosphorus content.

The efficiency of the untreated 200-mesh Florida pebble phosphate rock was superior or approximately equal to that of the superphosphate in the experiments with cabbage at the Alabama station and with millet at the West Virginia station. In the other experiments the efficiency of this material was decidedly inferior to that of superphosphate. The results of the two experiments in which direct comparisons were made indicate a slightly higher efficiency for the 200-mesh Florida pebble phosphate rock than for the 100-mesh material. The colloidal phosphate and the untreated Tennessee brown-rock phosphate were markedly less efficient than the untreated Florida pebble. The relative efficiency of dicalcium phosphate, as indicated by these experiments, was approximately the same as that reported by Ross, Jacob, and Beeson (*25*).

In the case of the calcined phosphates, removal of only 50 percent of the fluorine (sample 7) caused a reduction in the citrate solubility and a marked decrease in the relative nutrient efficiency of the phosphorus as compared with that of the phosphorus in the original phosphate rock. With further removal of fluorine there was a progressive increase in the citrate solubility and, with few exceptions, in the relative nutrient efficiency of the phosphorus. Calcined phosphates (samples 10 to 13) from which 90 percent or more of the total fluorine had been volatilized (corresponding to a citrate solubility of 65.4 percent or higher) showed higher average relative nutrient efficiencies than did superphosphate.

TABLE 4.—*Comparative efficiencies and availabilities of phosphates*

Sample no.	Phosphate	Average efficiency of P_2O_5 as indicated by increase in—		Availability of P_2O_5 determined by—	
		Dry weight of plants [1]	P_2O_5 content of plants [1]	Neutral ammonium citrate method [2]	2-percent citric acid method [2]
				Percent	*Percent*
1	Superphosphate	100.0	100.0	99.6	90.4
2	Dicalcium phosphate	[3] 112.4	[3] 110.9	100.0	100.0
3	Untreated Florida pebble phosphate, 200-mesh	[4] 66.6	[4] 67.1	13.2	21.9
4	Untreated Florida pebble phosphate, 100-mesh	[5] 38.5	[5] 28.6	11.6	21.9
5	Colloidal phosphate	[5] 26.7	[5] 22.8	10.4	31.7
6	Untreated Tennessee brown-rock phosphate	[6] 23.0	[6] 18.6	7.6	19.8
7	Calcined phosphate, prepared from Florida pebble	[4] 19.7	[4] 15.6	6.8	16.8
8	....do	[4] 54.9	[4] 44.5	20.6	27.7
9	....do	[4] 85.9	[4] 75.2	49.1	54.4
10	....do	[4] 109.4	[4] 104.5	78.1	82.6
11	Calcined phosphate, prepared from Tennessee brown rock	[4] 109.4	[4] 106.6	65.4	69.3
12	....do	[3] 116.2	[3] 125.9	86.1	92.7
13	....do	[7] 135.7	[7] 108.5	92.7	94.3

[1] Based on the increase from superphosphate as 100; excluding results obtained with limed soils.
[2] Based on the total P_2O_5 content of the material.
[3] Average of 7 series of experiments.
[4] Average of 6 series of experiments.
[5] Average of 2 series of experiments.
[6] Average of 3 series of experiments.
[7] 1 series.

COMPARATIVE EFFICIENCIES AND AVAILABILITIES OF PHOSPHATES

The data given in table 4 show a fairly direct relation between the availability of the phosphorus, as determined by either the neutral ammonium citrate or the 2-percent citric acid method, and the relative nutrient efficiency of the phosphorus, as indicated by the increase in either the dry weight or the phosphorus content of the plants. The relation is particularly noticeable in the series of calcined phosphates (samples 7 to 10) prepared from Florida pebble phosphate. The average relative efficiencies of the calcined phosphates were significantly higher than their solubilities in ammonium citrate and citric acid would indicate.

With the exception of superphosphate, the average efficiency of the phosphate materials was more closely correlated with the solubility of the phosphorus in 2-percent citric acid than in neutral ammonium citrate solution. Likewise, Jacob and Ross (*15*) report that the 2-percent citric acid method seems to give a better index of the nutrient

value of steamed bone meal, Tennessee brown-rock phosphate, and synthetic calcium phosphates than does the neutral ammonium citrate method. On the other hand, Bartholomew and Jacob (*2*) have shown that the citrate method gives, in general, a much better indication of the nutrient value of iron and aluminum phosphates than is obtained by the citric acid method. As compared with the citrate solubility, the lower citric acid solubility of the phosphorus in superphosphate (table 4; also, *14*, *15*) is probably due to the presence of iron and aluminum phosphates, either or both.

RELATION BETWEEN VOLATILIZATION OF FLUORINE AND NUTRIENT VALUE OF CALCINED PHOSPHATE

As compared with the untreated phosphate rock, volatilization of 50 percent of the total fluorine in the rock caused a decrease of approximately 40 percent in the nutrient value of the phosphorus (table 5)[6]. Also, the nutrient value of the phosphorus was decreased about 10 percent by the removal of 64 percent of the fluorine. On the other hand, volatilization of 97 percent of the total fluorine caused an increase of approximately 55 percent in the nutrient value of the phosphorus, as compared with an increase of about 35 percent when the rock was converted into superphosphate.

TABLE 5.—*Relation between volatilization of fluorine and nutrient value of calcined phosphate*

Sample no.	Phosphate material	Average nutrient value [1] of phosphate as indicated by total—		Fluorine volatilized in preparation of calcined phosphate	
		Dry weight of plants	P_2O_5 content of plants	Total fluorine	F'_B fluorine [2]
				Percent	*Percent*
3	Untreated Florida pebble phosphate, 200-mesh	100.0	100.0		
[3] 7	Calcined phosphate	62.7	59.2	49.9	0
[3] 8	do	93.1	85.3	64.2	1.6
[3] 9	do	123.1	116.3	78.9	44.0
[4] 11	do	144.8	143.1	[5] 90.0	[5] 76.0
[3] 10	do	143.3	142.0	93.3	82.2
[4] 12	do	149.6	163.9	[5] 97.0	[5] 93.0
1	Florida pebble superphosphate	134.3	137.9		

[1] Based on 100 as the total dry weight or P_2O_5 content of plants receiving untreated Florida pebble phosphate; excluding the second series of the Department of Agriculture.
[2] Fluorine corresponding to the second atom of fluorine in the fluorapatite equivalent of the total phosphorus.
[3] Prepared from Florida pebble phosphate.
[4] Prepared from Tennessee brown-rock phosphate.
[5] Approximate figure.

In the Florida pebble phosphate rock used in these experiments, the fluorine in excess of that corresponding to the second (F'_B) atom of fluorine in the fluorapatite equivalent of the total phosphorus, amounted to approximately 63 percent of the total fluorine. In agreement with the relation between fluorine volatilization and citrate solubility established in previous work (*17*, *22*, *23*), no increase in the nutrient value of the phosphorus occurred until all of the fluorine in excess of the F'_B fluorine was volatilized.

[6] It should be noted that the relative nutrient values shown in table 5 are based on the total dry weight and P_2O_5 content of the plants, in contrast to table 4 in which efficiency of P_2O_5 is based on increases of dry weight and P_2O_5 content.

The available evidence indicates that volatilization of fluorine in quantity less than that present in excess of the F'_B fluorine, results in the formation of hydroxyfluorapatite, a compound which is practically insoluble in citrate solution and which has comparatively little value as a source of phosphorus for plant growth. Volatilization of the F'_B fluorine results in the formation of hydroxyapatite, which also is comparatively insoluble in citrate solution. However, at 1,400° C. and in the presence of silica, the hydroxyapatite is converted into citrate-soluble phosphate, which is believed to be principally, if not entirely, the high-temperature form of tricalcium phosphate.

SUMMARY

In order to obtain information on the nutrient value of the phosphorus in calcined phosphate, greenhouse pot experiments with cabbage, millet, and Sudan grass were carried out on Cecil clay, Clarksville silt loam, Norfolk loamy fine sand, and Dekalb silt loam soil types, ranging in pH from 4.8 to 6.23. In the calcined phosphates, which were prepared from Florida land-pebble phosphate and Tennessee brown-rock phosphate, the citrate solubility of the phosphorus ranged from 7 to 93 percent, corresponding to the volatilization of 50 to 97 percent of the total fluorine. Comparative experiments were also made with superphosphate, dicalcium phosphate, and ground phosphate rock.

The nutrient value of calcined phosphate was related, more or less directly, to the citrate solubility of the phosphorus, which depended on the amount of fluorine volatilized from the rock during calcination. Volatilization of the fluorine in quantity less than that (about 63 percent of the total fluorine content of the phosphate rock) corresponding to the fluorine in excess of the second atom of fluorine in the fluorapatite equivalent of the total phosphorus, decreased the citrate solubility of the phosphorus, as compared with that of the phosphorus in the original phosphate rock, and markedly reduced the nutrient value of the phosphorus, as indicated by the plant growth and the absorption of phosphorus. Volatilization of 64 percent or more of the fluorine caused a progressive and pronounced increase in the citrate solubility and nutrient value of the calcined phosphate.

In general, calcined phosphates showing citrate solubilities of approximately 78 percent or higher (corresponding to the volatilization of 93 percent or more of the total fluorine content of the original phosphate rock) were as efficient sources of phosphorus for plant growth as were equivalent quantities of total phosphorus from either superphosphate or dicalcium phosphate.

In general, the effect of the phosphates in increasing the dry weight of cabbage, millet, and Sudan grass was related fairly closely to their effect in increasing the quantity of phosphorus absorbed by the plants.

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THE PHOSPHORUS CONTENT AND REQUIREMENTS OF THE FLOUR BEETLE TRIBOLIUM CONFUSUM DUVAL, AND A STUDY OF ITS NEED FOR VITAMIN D[1]

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INTRODUCTION

Little is known regarding the mineral requirements of insects or their mineral content. Loeb (*4*)[2] demonstrated that *Drosophila* requires phosphorus but not calcium. Uvarov (*11*) summarized the existing knowledge of the phosphorus content of insects, nine species only being listed. *Tribolium* was not mentioned. Sweetman and Palmer (*10*) showed the feasibility of using *Tribolium* as a test animal for biological analysis. They found that this insect requires vitamin B (undifferentiated complex) in its food, as had been demonstrated for *Drosophila* by Guyénot (as cited by Uvarov (*11*)), Northrop (*6*), and Bacot and Harden (*1*), and for *Ephestia kuehniella* by Richardson (*9*). Hobson (*2*) showed that aseptic blowfly larvae require four types of growth factors found in yeast. These factors are an insoluble fraction and three soluble fractions, one heat-labile, probably B_1, and two heat-stable, probably Y and B_2. No need of *Tribolium* for vitamin A could be demonstrated by Sweetman and Palmer, and additions of vitamin D to a normal ration exerted no beneficial effect. However, no experiments have been reported that show whether insects actually do require vitamin D.

MATERIAL AND METHODS

Tribolium confusum was employed in a study of phosphorus requirement, and inasmuch as the normal utilization of this element by vertebrates is greatly influenced by vitamin D, advantage was taken of the opportunity to examine more critically the question as to whether this insect requires vitamin D. The adequacy of the rations tested was measured in a constant physical environment by the length of the larval stage, either from the day-old egg or from hatching to pupation.

In order to secure a sufficient number of individuals for phosphorus analysis, day-old eggs were placed directly in the experimental rations, which had been adjusted to 10 percent moisture, and incubated at a temperature of 32° C. and a relative humidity of 75 percent. At the time of pupation the pupae were removed daily by sifting the culture through a coarse sieve. About 200 live larvae, pupae, or adults and about 4,000 eggs (figuring 450 per 0.1 cc) represented a single sample for analysis. Dry-matter determinations were made at 100° C. under reduced pressure in vacuo, and total ash, calcium, and phosphorus were determined by the method of Morris, Nelson, and Palmer (*5*).

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[2] Reference is made by number (italic) to Literature Cited, p. 852.

EXPERIMENTAL RESULTS

Preliminary experiments had indicated that the adults contained more phosphorus than the pupae, and that this increase was taking place in the pupae before metamorphosis was completed.

The results of the analyses of all the stages, given in table 1, show that the percentage of dry matter decreases with advancing stages in the life cycle and that the percentage of ash and phosphorus in the dry matter increases.

Table 2 gives the results of growing *Tribolium* on irradiated and nonirradiated rations of different sorts. When irradiated and nonirradiated farina (with and without added $CaCO_3$) were used, no differences in the growth of *Tribolium* were observed. When a synthetic irradiated diet was employed the time required for pupation was greater than when a nonirradiated diet was used. Adding cod-liver oil to a nonirradiated ration decreased the time of pupation slightly, but not significantly. The slight benefit of cod-liver oil was more likely due to the alleviation of a dusty condition of this ration than to its vitamin content.

TABLE 1.—*Mean analysis of different stages in the life cycle of Tribolium confusum when kept at a temperature of 32° C. and a relative humidity of 75 percent, whole-wheat flour, sifted through no. 6 bolting cloth, being used as food*

Stage	Individual live weight	Dry matter	Content on a dry-matter basis of—		
			Ash	Calcium	Phosphorus
	Milligrams	*Percent*	*Percent*	*Percent*	*Percent*
Egg	0.092	58.80	1.85	0.024	0.445
Larval		42.26	2.07	.040	.456
Pupal	2.0	40.01	2.15	.066	.522
Adult		39.07	2.36	.054	.598

TABLE 2.—*Growth of Tribolium confusum on rations containing or deficient in vitamin D with and without a calcium supplement, when kept at a temperature of 32° C. and a relative humidity of 75 percent*

[Approximately 1,100 day-old eggs were placed in 300 g of food]

Ration	Phosphorus in ration	Calcium in ration	Pupae	Period of growth, egg to pupation	
				Mean [1]	σ [2]
	Percent	*Percent*	*Number*	*Days*	
Irradiated farina [3]	0.097	0.030	262	51.5±0.429	10.3±0.303
Nonirradiated farina	.097	.030	338	51.3± .348	9.5± .246
Irradiated farina+1.5 percent $CaCO_3$	.099	.644	243	56.5± .407	9.4± .288
Nonirradiated farina+1.5 percent $CaCO_3$	.099	.644	227	56.1± .145	3.2± .102
Irradiated synthetic diet [4]	.196	.527	268	74.1± .330	8.2± .240
Nonirradiated synthetic diet [4]	.196	.527	367	60.8± .214	6.1± .151
Nonirradiated synthetic diet [4]+0.5 percent cod-liver oil	.196	.527	504	55.0± .182	6.1± .129

[1] Mean $=\bar{x}=\frac{\Sigma x}{N}$; ±Probable error $\bar{x}=0.6745\ \frac{\sigma x}{\sqrt{N}}$.

[2] σ=Standard deviation $=\sqrt{\frac{\Sigma x^2}{N}-x^2}$; ±Probable error $\sigma_x=0.6745\ \frac{\sigma x}{\sqrt{2N}}$.

[3] Ration irradiated for 15 minutes at 20 inches with a quartz mercury vapor sun lamp, operating at 110 volts, 5 amperes.

[4] Synthetic diet contained 12.5 percent arachin, 2.5 percent edestin, 3 percent Crisco, 75.5 percent dextrin, 4 percent Osborne and Mendel (7) salt mixture with only one-half the normal amount of phosphorus present, and 2.5 percent of yeast extract made by digesting pure dry yeast with 50-percent ethyl alcohol for 2 days at 32° C. The arachin was prepared from defatted peanut meal by the method of Johns and Jones (3) and the edestin from defatted hempseed by the sodium benzoate method of Reeves (8).

Inasmuch as the lowest phosphorus content of these rations provep to be essentially the limiting level of this element it is evident that *Tribolium* does not have a vitamin D requirement analogous to that of the vertebrates. The similar growth on similar rations of the high calcium low phosphorus type, on which rats would become so rachitic unless vitamin D is provided, lends further support to this conclusion.

The phosphorus requirement of *Tribolium* was determined from the growth and phosphorus content of the insect when reared on a synthetic ration containing five definite levels of phosphorus. These levels were obtained by using the synthetic ration described in table 2 and five salt mixtures prepared by the Osborne and Mendel (7) technic, one being phosphorus free. From the results given in table 3 it is seen that under the conditions described the time of pupation was proportional to the amount of phosphorus present in the ration. The limiting amount of phosphorus is probably about 0.1 percent. The difference between the time of pupation on 0.1- and 0.2-percent levels of phosphorus was 35 days, which was 248 times the probable error of the difference. However, there was no significant difference in the amount of phosphorus in the pupae. The data indicate that the larvae must eat sufficient food to bring their phosphorus content up to a certain level before pupation will occur. This requirement for phosphorus probably explains in part the slower development and smaller populations of *Tribolium* in patent flour and similar products than in whole-wheat flour. Patent flour ranges in phosphorus content from 0.07 to 0.10 percent.

TABLE 3.—*Growth and phosphorus content of Tribolium confusum on synthetic rations with different phosphorus levels, when kept at a temperature of 32° C. and a relative humidity of 75 percent*

[Approximately 8,100 day-old eggs were placed in 1,500 g of rations 1, 4, and 5, 2,700 in 500 g of ration 2, and 5,400 in 848 g of ration 3]

Ration [1]	Phosphorus content	Pupae	Period of growth, egg to pupation		Phosphorus in dry pupae	
			Mean	σ	Mean	σ
	Percent	*Number*	*Days*		*Percent*	
1	0.017	995	89.96±0.387	18.09±0.274	0.517±0.003	0.009±0.002
2	.107	775	71.49± .138	5.71± .098	.539± .002	.006± .001
3	.205	4,346	35.95± .038	3.72± .027	.530± .002	.015± .002
4	.296	6,881	34.25± .029	3.60± .021	.535± .001	.012± .001
5	.336	7,335	33.43± .026	3.27± .018	.540± .001	.011± .001

[1] Synthetic ration the same as in table 2, the only variable being the amount of phosphorus in the salt mixture.

SUMMARY AND CONCLUSIONS

Analyses of *Tribolium confusum* Duval at different stages in its life cycle show that the eggs, larvae, pupae, and adults contain, respectively, 58.8, 42.26, 40.01, and 39.07 percent of dry matter. The dry matter of eggs, larvae, pupae, and adults contains, respectively, 1.85, 2.07, 2.15, and 2.36 percent of ash, and 0.445, 0.456, 0.522, and 0.598 percent of phosphorus.

Tribolium does not need vitamin D for any physiologic function that can be detected by length of time to pupation or by phosphorus content of the pupae.

The time of pupation of *Tribolium* is somewhat proportional to the amount of phosphorus in the ration, other factors being constant. The limiting amount of phosphorus is probably 0.1 percent. However, the percentage of phosphorus in the pupae is constant regardless of the amount of phosphorus in the ration.

The slower development and smaller populations of *Tribolium* in patent flour and similar products than in whole-wheat flour is probably due in part to the low phosphorus content of these foods.

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VITAMIN A CONTENT OF EGGS PRODUCED BY CHICKENS FED VIOSTEROL AND VARIOUS PERCENTAGES OF COD-LIVER OIL[1]

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INTRODUCTION

Very little is known regarding the interrelationships of any of the vitamins either in man or in experimental animals. Much of what little work has been done has given results of a negative nature. Coward (*3*),[2] however, has recently reported data which apparently indicate that with rats the growth responses to different doses of one vitamin are influenced by the amount of another vitamin present in the diet. In proof of this she shows that curves of response—average gain in weight plotted against units of vitamin D fed—obtained by giving the same series of doses of vitamin D but with basal diets containing different amounts of vitamin A do not coincide at any point. Similar findings are reported for the B vitamins.

Recent work in this laboratory has shown that the vitamin D content of eggs may be increased many times by adding viosterol to the diet of the chicken (*Gallus domesticus*). It is common knowledge that the vitamin A content of eggs depends upon the amount of this factor in the diet of the chicken. In view of the possibility that vitamin D in the form of viosterol might have some effect on the vitamin A storage in the eggs, it seemed advisable, before definitely recommending that poultry raisers use viosterol to increase the vitamin D content of eggs, to determine what effect, if any, this added vitamin D might have on the vitamin A content of the egg. Cod-liver oil was used in the diet as the source of vitamin A. Incidental to this study of vitamin interrelationships data were obtained on the influence of the various levels of cod-liver oil in the diet of the chicken on vitamin A content of egg yolk.

REVIEW OF LITERATURE

Data on the vitamin A content of eggs have been reported by various workers. Sherwood and Fraps (*9*) have recently shown that eggs from pullets fed a basal diet containing 20 percent yellow corn as the only source of vitamin A had about 20 Sherman-Munsell units of vitamin A per gram of yolk at the beginning of the experiment and from 5 to 8 toward the end of the 6½-month feeding period.

Bisbey and coworkers (*2*) reported the influence of yellow corn, and yellow corn plus alfalfa or cod-liver oil in the diet of the chicken on the vitamin A content of the egg yolk. The percentage of these components in the diets and the vitamin A content of the egg yolk

[1] Received for publication Mar. 7, 1935; issued July, 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 859.

were as follows: On 65 percent of yellow corn plus 10 percent of alfalfa, 28 units (Sherman-Munsell) per gram of yolk; on 65 percent of yellow corn, 9 units; on 35 percent of yellow corn, 7 units; and on 25 percent of yellow corn plus 0.5 percent of cod-liver oil, 28 units.

Munsell (*7*) reported the vitamin A content of some eggs purchased on the New York market as 50 units per gram of yolk.

Bethke, Kennard, and Sassaman (*1*) gave the vitamin A value of yolks of eggs from confined birds as varying from 5.2 to 11.4 units, while that of eggs from birds on range or receiving cod-liver oil had 25 units or more per gram.

MATERIALS AND METHODS

Three pairs of pens of crossbred pullets kept at the United States Animal Husbandry Farm at Beltsville, Md., were used in the present experiment. These birds were the offspring resulting from mating Barred Plymouth Rock females with Rhode Island Red males. Each pen contained 18 pullets and 1 cockerel. The birds were confined indoors and received no direct sunlight. A description of the basal diet and the vitamin supplements are given below.

Basal diet fed to paired pens of pullets

	Percent		Percent
Basal feed mixture	73. 15	Ground limestone	2. 87
Desiccated meat meal	8. 00	Special steamed bone meal	2. 98
North Atlantic whitefish meal	7. 00	Anhydrous Na_2SO_4	. 50
Dried buttermilk	5. 00	NaCl	. 50

Vitamin supplements given to paired pens of pullets

Pen 21—1 percent of cod-liver oil.
Pen 22—1 percent of cod-liver oil and 0.5 percent of viosterol 160 D.
Pen 23—2 percent of cod-liver oil.
Pen 24—2 percent of cod-liver oil and 0.5 percent of viosterol 160 D.
Pen 25—8 percent of cod-liver oil.
Pen 26—8 percent of cod-liver oil and 0.5 percent of viosterol 160 D.

The basal feed mixture which made up the bulk of the basal diet contained yellow corn meal, 52.63 percent; wheat bran, 25.79 percent; rolled oats, 15.79 percent; alfalfa-leaf meal, 5.79 percent. The cod-liver oil was a high-grade commercial product sold for animal-feeding purposes. The pullets were put on the above-described diets at the end of August 1933. Egg samples for analysis were taken from January 1 through June. Each sample was made up from the eggs laid during a 7-day period and included one egg from each pullet that laid.

In addition to eggs from pullets in the 6 pens listed above, a composite sample of eggs from 8 pens of pullets fed diets which contained no added cod-liver oil or viosterol was tested for vitamin A. The diets fed to these 8 pens of pullets contained the same percentage of basal feed mixture as the basal diet described above but the protein supplements in each case were entirely of plant origin. The birds on these diets had access to a limited range. The eggs will be referred to as from pullets on a. p. s. (all plant source) diet.

Vitamin A tests were made according to the regular Sherman-Munsell method (*8*) except that the test period was of 5 weeks' duration instead of the usual 8 weeks. The basal diet fed to the rats

was a slight modification of that used by Sherman and Munsell. It contained the following ingredients: Vitamin-A-free casein, 18 percent; irradiated yeast, 5 percent; nonirradiated yeast, 5 percent; Osborne and Mendel salts, 4 percent; sodium chloride, 1 percent; hydrogenated vegetable fat, 10 percent; and starch, 57 percent. The rats were 21 days old when put on the basal diet. They showed cessation of growth and quite uniform ophthalmic symptoms about the thirty-first day on this diet.

Samples of egg-yolk mixture for testing were made up each week. The yolks were separated as cleanly as possible from the whites and any small amount of white still adhering was removed with filter paper. The yolks were then mixed thoroughly with an electric beater and a weighed portion of the composite sample mixed with four times its weight of vitamin-A-free casein. These egg-casein mixtures were weighed out on an analytical balance three times weekly, and fed as supplements to the basal diet. Between weighings they were stored in air-tight containers at 20° F.

EFFECT OF VIOSTEROL

The main purpose of the experiment was to determine whether there was any difference in vitamin A storage in the eggs due to added vitamin D in the diet. Therefore equal weights of yolk of eggs from pullets given cod-liver oil and from those given the same quantity of cod-liver oil with viosterol were allotted to litter-mate test animals of the same sex and weight. For example, when a rat was allotted 0.05 g per day of yolk of eggs from pen 21 (table 1) a carefully matched animal of the same litter, sex, and weight was allotted the same amount of yolk of eggs from pen 22.

TABLE 1.—*Comparison of the gain in weight and the food intake of paired groups of rats having as the only source of vitamin A the yolk of eggs from pullets receiving cod-liver oil or cod-liver oil and viosterol*

Pairs of rats fed egg yolk (number)	Quantity of egg yolk fed per rat per day	Eggs obtained from pen no.—	Supplement in diet of pullets		Total gain in weight of rats during 5-week test period			Total food intake of rats		
			Cod-liver oil	Viosterol 160 D	Average per rat	Standard deviation of differences between pairs of rats	Probability that differences may have occurred by chance	Average per rat	Standard deviation of differences between pairs of rats	Probability that differences may have occurred by chance
	Grams		*Pct.*	*Percent*	*Grams*			*Grams*		
10	0.0125	21	1	0	(1)					
		22	1	.5						
10		23	2	0	(1)					
14		24	2	.5						
		25	8	0	19.9	22.96	0.47	241.4	48.96	0.65
		26	8	.5	15.3			235.4		
10	.025	21	1	0	6.2	19.93	.15	219.7	34.95	.10
		22	1	.5	16.3			240.2		
11		23	2	0	21.2	13.10	.53	254.0	29.47	.41
15		24	2	.5	18.7			246.1		
		25	8	0	48.8	14.65	.02	297.1	31.61	.19
		26	8	.5	38.9			285.8		
17	.05	21	1	0	28.2	15.48	.86	263.3	32.59	.32
		22	1	.5	27.5			271.4		
14		23	2	0	34.4	17.18	.89	276.4	46.30	.80
15		24	2	.5	35.0			279.6		
		25	8	0	52.9	15.51	.63	304.7	35.98	.63
		26	8	.5	54.9			309.3		

[1] 13 of the 20 rats died before the end of the 5-week test period.

The egg samples were also allotted to the members of a litter of test animals in such a way that all (up to six) received the same weight of egg mixture but each from a different pen. Therefore comparisons of the growth of animals on the same weight of egg from the various diets are comparisons, for the most part, between litter mates, while those between rats fed different amounts of the same kind of egg are not litter-mate comparisons. Furthermore, the eggs were apportioned in such a way that any seasonal influence would be equalized in the tests of all of the different kinds of eggs and the different levels of feeding.

Feeding trials of this kind with several pairs of rats were made at three levels, 0.0125, 0.025, and 0.05 g. Most of the animals fed 0.0125 g daily of yolk of eggs from pens 21, 22, 23, and 24 died before the end of the 5-week test period. Therefore no data were available for analysis at this level of the eggs from these two pairs of pens.

The effect of added vitamin D in the diet of the chicken on the vitamin A content of the egg yolk was evaluated by comparing the differences between the gains in weight made by the rats of each pair by Student's method as described by Fisher (*5*). The formula used is

$$t=\frac{\text{mean difference} \times \sqrt{N}}{S},$$

where S is the observed standard deviation of the differences and N is the number of pairs. A t value corresponding to a probability of 0.01 is to be considered as indicating a significant average difference. The mean gains, food intake, number of pairs used in each comparison, the standard deviation, and the probability of getting a difference as great or greater than the observed difference are tabulated in table 1.

Since only one of the differences has a probability that approaches 0.01 and the rest are greater than 0.05, it may be concluded that the presence of added vitamin D in the diet of the chicken had no significant influence on vitamin A storage in the eggs.

EFFECT OF DIFFERENT LEVELS OF COD-LIVER OIL

Comparisons were also made of the vitamin A content of the yolks of eggs from pullets on the various levels of cod-liver oil and those on the all-plant-source diets. Data from rats fed the same weight of egg yolk from the two groups of pullets on each level of cod-liver oil were combined irrespective of whether the diet contained added viosterol. For example, the data from rats fed 0.025 g of yolk of eggs from pen 21 were combined with the data from rats receiving a like amount of yolk of eggs from pen 22. This seemed a logical procedure since no effect on storage of vitamin A in eggs due to viosterol in the diet of the pullet could be demonstrated. A few additional values were included that were not used in the paired comparisons of the first analysis either because the animal from which the data were obtained was an odd one of a litter or because its litter mate had died. Each group, however, contained equal numbers of males and females.

These comparisons were made by covariance analysis. The gains in weight were corrected for differences in feed intake by means of the regression of gain in weight on feed eaten. Snedecor (*10*) and Tippett

(*11*) describe the mechanics of this method. Applications of covariance analysis to biological data are given in detail by Titus and Harshaw (*12*) and by Crampton (*4*).

As already explained, data were available from only seven of the groups of rats fed eggs from pullets given cod-liver oil. These 7 groups as well as the 2 groups fed eggs from pullets given no added vitamin concentrate were regarded as separate treatments. An analysis of covariance was considered justifiable since the slopes of the regression lines expressing the relation between feed and gain were approximately the same for all of the nine groups. The variance was divided only into that attributable to treatment and to error. Using the pooled regression coefficient of 0.33204, the sums of squares were corrected and a z value [$z=\frac{1}{2}$ ($\log_e$ variance due to treatment $-$ $\log_e$ variance due to error)] of 1.1188 was obtained. The necessary z for $P=0.01$ when $n_1=8$ and $n_2=254$, is 0.4742. Since the calculated value of z is greater than this, it is probable that there are significant differences among the corrected gains which are not attributable to chance. The mean gain in weight of the rats of each group was then corrected by subtracting the product of the regression coefficient and the difference between the mean food intake of the group and the mean food intake of all of the groups (266.8 g). The corrected values are given in table 2.

TABLE 2.—*Observed and corrected average gains in weight of the groups of rats used in the vitamin A tests on yolk of eggs from pullets fed different percentages of cod-liver oil*

Rats in group (number)	Quantity of egg yolk fed per rat per day as source of vitamin A	Eggs obtained from pen no.—	Cod-liver oil in diet of pullets	Average gain in weight per rat (5 weeks)	Average food intake per rat (5 weeks)	Average corrected gain in weight per rat (5 weeks)
	Grams		*Percent*	*Grams*	*Grams*	*Grams*
28	0.0125	25–26	8	17.6	238.6	26.9
30	.025	21–22	1	11.3	231.4	23.1
30		23–24	2	19.5	244.7	26.8
38		25–26	8	44.1	292.1	35.7
36	.050	21–22	1	29.2	268.7	28.5
38		23–24	2	34.4	274.7	31.8
30		25–26	8	54.1	306.4	40.9
18	.050	(1)	0	19.0	246.6	25.7
16	.10	(1)	0	43.7	289.0	36.3

[1] All-plant-source diet.

In table 3, treatment differences between any two groups are summarized. The significance of the differences was evaluated by means of the t test. A t value corresponding to a probability of 0.05 is considered significant and 0.01 highly significant. The number of degrees of freedom (n) is 254, since that number was used in estimating the error variance.

The results of the analysis may be summarized briefly. The eggs from birds fed 1 and 2 percent of cod-liver oil were significantly different in vitamin A content from those fed 8 percent. Since an equivalent weight of the yolks of eggs from the latter pair of pens produced gains in weight uniformly greater than an equal amount of yolk of eggs from either of the other two pairs of pens, it is concluded that the eggs from the birds fed 8 percent of cod-liver oil were richer

in vitamin A than the others. Additional evidence of the greater vitamin A content of the eggs from birds on the high cod-liver oil diets was the survival of most of the rats fed the 0.0125-g portion of the yolks of eggs from these pens and the fact that the majority of the rats fed the same amount of yolk from the other two pairs of pens died. No significant difference in vitamin A content was found between the eggs from the two groups fed 1 and 2 percent of cod-liver oil. However, since differences in gains in weight of the test animals fed equal quantities of egg yolk were always positive in favor of the pullets receiving 2 percent of cod-liver oil, it is probable that if larger numbers of animals had been used in making the vitamin tests a significant difference between these two levels of cod-liver oil might have been demonstrated. The eggs from the birds fed no vitamin A supplement were significantly different in vitamin A content from those of the birds fed cod-liver oil. Since the gains in weight of the rats fed eggs from the birds receiving no cod-liver oil were uniformly inferior it is concluded that these eggs were poorer in vitamin A than any of the others.

TABLE 3.—*Significance of differences* [1] *in average gains in weight of groups of rats having as the sole source of vitamin A yolk of eggs from pullets fed different percentages of cod-liver oil*

Quantity egg yolk per rat per day (grams)	Eggs obtained from pen no.—	Results when given quantity of egg yolk from indicated pen number was fed per rat per day								
		0.0125 g	0.025 g			0.050 g			0.05 g	0.10 g
		25–26	21–22	23–24	25–26	21–22	23–24	25–26	A.p.s.[2]	A.p.s.[2]
0.0125	25–26		+N	+N	−H	−N	−S	−H	+N	−H
0.025	21–22	−N		−N	−H	−S	−H	−H	−N	−H
	23–24	−N	+N		−H	−N	−S	−H	+N	−H
	25–26	+H	+H	+H		+H	+N	−S	+H	−N
0.050	21–22	+N	+S	+N	−H		−N	−H	+N	−S
	23–24	+S	+H	+S	−N	+N		−H	+S	−N
	25–26	+H	+H	+H	+S	+H	+H		+H	+N
0.050	(3)	−N	+N	−N	−H	−N	−S	−H		−H
0.10	(3)	+H	+H	+H	+N	+S	+N	−N	+H	

[1] A difference that can be expected to occur by chance only 1 in 100 times is called highly significant and is indicated by the letter H; one that may occur only 1 in 20 times is called significant, S; and one that would not occur by chance more often than 1 in 20 times is called not significant, N. A positive sign at the side of a letter indicates that the difference is in favor of the pen listed at the left; a negative sign indicates that the difference is in favor of the pen listed in the box head at the top of column.

[2] All-plant-source diet.

TABLE 4.—*Vitamin A content of yolk of eggs from pullets receiving different percentages of cod-liver oil or cod-liver oil and viosterol*

Eggs obtained from pen no.—	Supplement in diet of pullets		Vitamin A units per gram of egg yolk (Sherman-Munsell units)	Eggs obtained from pen no.—	Supplement in diet of pullets		Vitamin A units per gram of egg yolk (Sherman-Munsell units)
	Cod-liver oil	Viosterol 160 D			Cod-liver oil	Viosterol 160 D	
	Percent	*Percent*	*Number*		*Percent*	*Percent*	*Number*
21	1	0	(1)	25	8	0	80
22	1	.5	(1)	26	8	.5	80
23	2	0	40	A. p. s.[2]	0	0	20
24	2	.5	40				

[1] Slightly less than 40.

[2] All-plant-source diet.

In table 4 are listed the approximate number of Sherman-Munsell units of vitamin A in the yolks of the eggs from the various sources.

CONCLUSIONS

Vitamin D in the form of 0.5 percent of viosterol 160 D in the diet of the chicken (*Gallus domesticus*) receiving graded quantities of cod-liver oil had no apparent effect on the transfer of vitamin A to the egg.

Eggs from pullets fed 8 percent of cod-liver oil were several times richer in vitamin A than eggs from pullets fed 1 and 2 percent of cod-liver oil. These findings should not necessarily be interpreted to mean that in ordinary poultry practice it is desirable to increase the cod-liver oil content of the diet to an 8-percent level in order to increase the vitamin A content of the eggs. It has been definitely shown (*6*) that the optimum level of cod-liver oil feeding in the diet of the chicken is between 1 and 2 percent. When this level is exceeded impairment in egg production and hatchability is likely to occur.

Although no significant difference could be demonstrated between eggs from birds fed 1 and 2 percent of cod-liver oil, it is probable that had more test animals been used a significant difference in favor of the 2-percent group might have been shown.

The vitamin A potency of eggs from pullets on diets containing no vitamin A and vitamin D supplement was significantly less than the potency of eggs from any of the groups given cod-liver oil. The eggs from these pullets were judged to be about one-fourth as potent as eggs from birds fed 8 percent of cod-liver oil and about one-half as potent as those from birds fed 1 or 2 percent of cod-liver oil. The eggs from birds receiving no vitamin A and B supplement had about 20 units (Sherman-Munsell) of vitamin A per gram of egg yolk, while the eggs from birds fed 8 percent of cod-liver oil had about 80 units, and those from pullets fed 1 or 2 percent of cod-liver oil about 40 units.

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